Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy

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Abstract

Objective: Because the secretion of dehydroepiandrosterone (DHEA), the exclusive source of sex steroids in postmenopausal women, is already decreased by 60% and continues to decline at the time of menopause, the objective of this study was to examine the effect of intravaginal DHEA on the symptoms and signs of vaginal atrophy.

Methods: This prospective, randomized, double-blind and placebo-controlled phase III clinical trial studied the effect of Prasterone (DHEA) applied locally in the vagina on the signs and symptoms of vaginal atrophy in 216 postmenopausal women.

Results: All three doses (0.25%, 0.5%, and 1.0%) of DHEA ovules applied daily intravaginally induced a highly significant beneficial change in the percentage of vaginal parabasal and superficial cells and pH as well as in the most bothersome symptom at 2 weeks. At the standard 12-week time interval, 0.5% DHEA caused a 45.9 ± 5.31 (P < 0.0001 vs placebo) decrease in the percentage of parabasal cells, a 6.8 ± 1.29% (P < 0.0001) increase in superficial cells, a 1.3 ± 0.13 unit (P < 0.0001) decrease in vaginal pH, and a 1.5 ± 0.14 score unit (P < 0.0001) decrease in the severity of the most bothersome symptom. Similar changes were seen on vaginal secretions, color, epithelial surface thickness, and epithelial integrity. Comparable effects were observed at the 0.25% and 1.0% DHEA doses.

Conclusions: Local Prasterone, through local androgen and estrogen formation, causes a rapid and efficient reversal of all the symptoms and signs of vaginal atrophy with no or minimal changes in serum steroids, which remain well within the normal postmenopausal range. This approach avoids the fear of systemic effects common to all presently available estrogen formulations and adds a novel physiological androgenic component to therapy.

Key Words: Vaginal atrophy – Dehydroepiandrosterone – Prasterone – Intracrinology – Androgens – Tissue-specific prehormone replacement therapy.

Vaginal dryness is found in 75% of postmenopausal women.1,2 For various reasons, especially the fear of adverse effects caused by estrogens, only 20% to 25% of symptomatic women who have vaginal atrophy seek medical treatment.3,4 There is thus a clear medical need and a major opportunity to remove the fear of breast cancer associated with therapy while improving the quality of life of most women who are left with the problem of vaginal atrophy for a large proportion of their lifetime. It can be mentioned that although hot flashes abate spontaneously with time without treatment, vaginal atrophy symptoms, namely, vaginal dryness, vulvovaginal irritation/itching, and pain at sexual activity, usually increase in severity with time in the absence of treatment.

Based on the knowledge that estrogen secretion by the ovaries ceases at menopause in all women, systemic and local estrogens have so far been the traditional and exclusive treatment for vaginal atrophy. A series of reports have indicated, however, that systemic estrogens + progestin (hormone therapy) and estrogens alone (estrogen therapy) increase the risk of breast4,10 ovarian,5,6,11,12 and endometrial (estrogens alone)13,14 cancer. The publicity that followed knowledge by
TABLE 1. Laboratory tests evaluating the hematology, coagulation, blood chemistry, and urinalysis parameters

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematology</td>
<td>WBC, RBC, Hemoglobin, Hematocrit, Platelets, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Bands, Fibrogen, Prothrombin, Partial thromboplastine time, Albumin, Alkaline phosphatase, AST (SGOT), ALT (SGPT), Total bilirubin, Blood urea nitrogen (BUN), Calcium, Chloride, Creatinine, Glucose, Inorganic phosphorus, LDH, Magnesium, Potassium, Total protein, Sodium, Uric acid, Color, Specific gravity, pH, Glucose, Ketones, Blood, Protein, Nitrites, Leukocytes, Microscopic RBC, Microscopic WBC, Bacteria, Epithelial cells, Casts, Crystals</td>
</tr>
<tr>
<td>Coagulation</td>
<td></td>
</tr>
<tr>
<td>Blood chemistry</td>
<td></td>
</tr>
<tr>
<td>Urinalysis</td>
<td></td>
</tr>
</tbody>
</table>

WBC, white blood cells; RBC, red blood cells; AST, aspartate aminotransferase; SGOT, serum glutamic-oxaloacetic transaminase; ALT, alanine aminotransferase; SGPT, serum glutamic-pyruvic transaminase; LDH, lactate dehydrogenase.

Because serum dehydroepiandrosterone (DHEA) is the almost exclusive source of androgens, which play important physiological roles in women, the 60% decrease in circulating DHEA already found at time of menopause is accompanied by a similar 60% decrease in the total androgen pool. Such a marked decrease in androgens can potentially lead to signs and symptoms of hypoandrogenicity in the bone, muscle, skin, mammary gland, vagina, and brain as well as on glucose, insulin, and lipid metabolism.

Among the androgen target tissues, recent preclinical data obtained in experimental animals have clearly shown beneficial effects of the androgens made locally from DHEA in the vagina, not only in the superficial epithelial layer but also importantly on collagen fibers of the lamina propria and on the muscularis. These data clearly indicate the importance of androgens for normal vaginal physiology, a role that cannot be achieved with estrogens.

Based on the data of both our preclinical and clinical studies showing beneficial effects on the vagina of DHEA administered percutaneously or locally, the present clinical trial is a prospective, randomized, and placebo-controlled study of the effect of four doses of intravaginal DHEA administered daily for 12 weeks on the changes in vaginal superficial and parabasal cells as well as on vaginal pH and on the most bothersome symptoms of vaginal atrophy as coprimary objectives. The data clearly show that locally administered DHEA is very efficient and rapid in correcting the signs and symptoms of vaginal atrophy, a near maximal effect being already achieved at 2 weeks.

METHODS

This study was a phase III, prospective, multicenter, randomized, placebo-controlled, and double-blind trial planned for 50 participants per arm (for a total of 200 participants to complete the study). Two hundred eighteen postmenopausal women (216 women in the intent-to-treat [ITT] analysis) were thus randomized to receive a daily ovule of the following DHEA concentrations: 0.0% (53 women), 0.25% (3.25 mg DHEA; 53 women), 0.5% (6.5 mg DHEA; 56 women), or 1.0% (13 mg DHEA; 54 women) applied intravaginally with an applicator at bedtime. Two hundred sixteen women were included in the ITT population, which served as the basis of the analysis. The median age of women was 58 years (range, 49-70 y), 57 years (range, 42-72 y), 58 years (range, 50-74 y), and 59 years (46-69 y) in the four groups. The DHEA ovules or suppositories (Vaginorm) containing Prasterone in a lipophilic base were manufactured by Recipharm (Karlskoga, Sweden). The study was divided into two phases, namely, screening followed by a treatment period of 12 weeks. The protocol was approved by the institutional review board of the Centre Hospitalier de l’Université Laval, Quebec City, QC, Canada; McGill University, Montreal, QC, Canada; Ethica, Montreal, QC, Canada; Eastern Virginia Medical School, Norfolk, VA; and the Western Institutional Review Board, Los Angeles, CA.

Although intravaginal formulations were developed to avoid systemic exposure to estrogens, a series of studies have unanimously demonstrated that all intravaginal estrogen formulations lead to significant increases in serum estrogen levels measured directly by radioimmunoassay or through their systemic effects. Recent data obtained with the most accurate technology indicate that the effects of estrogens applied locally in the vagina are unlikely to be limited to the vagina and that systemic action is expected, as previously suggested.
The inclusion criteria were the following:
- Postmenopausal women who satisfy a or b or c:
  a. No menses for at least 1 year; or
  b. Follicle-stimulating hormone levels of 40 mIU/mL or more (within 60 d before day 1) in women with no menses for 6 months or more but less than 12 months, or hysterectomized women who were premenopausal at the time of hysterectomy; or
  c. 6 weeks or more (of screening visit) after bilateral oophorectomy.
- Women having self-identified at least one moderate to severe of the following symptoms:
  • Vaginal dryness (none, mild, moderate, or severe);
  • Vaginal and/or vulvar irritation/itching (none, mild, moderate, or severe);
  • Vaginal pain associated with sexual activity (none, mild, moderate, or severe).
Women were asked to identify which symptom is the most bothersome to her at the start of treatment. The change in this symptom was followed and served to evaluate the effect of treatment.
- Women between 40 and 75 years of age
- Women willing to participate in the study and sign an informed consent form
- Women having a low maturation index (no greater part of guidance than 5% of superficial cells on vaginal smear)
- Women having a vaginal pH above 5
- Women having a normal mammogram within 9 months of study start
- Women having a normal breast examination
- Women having normal Papanicolaou (Pap) smear (which includes inflammatory changes) within the previous 12 months of day 1 (for hysterectomized women, the Pap smear was done with at least one slide)
- Women having no former or present narcotic addiction or alcoholism
- Women having body weight within the range of 18.5 to 35 kg/m² of ideal body weight according to body mass index (World Health Organization)
- Women having no hepatic or renal impairment or condition known to affect drug or steroid metabolism
- Women having normal baseline hematology, clinical chemistry, and urinalysis results
- Women having negative serology results for HIV-1/HIV-2 and hepatitis B and C.

The exclusion criteria were as follows:
- Undiagnosed abnormal genital bleeding
- Previous diagnosis of cancer, except skin cancer (non-melanoma)
- Endometrial hyperplasia at biopsy performed at screening or endometrial cancer
- Active or history of thromboembolic disease
- Significant metabolic or endocrine disease
- Clinically significant gastrointestinal, liver, or gallbladder disease
- Recurrent migraine headache not controlled by conventional therapy
- Diabetes mellitus not controlled by conventional therapy
- Significant complication on previous hormone therapy
- Use of estrogen-alone injectable drug therapy or progestin implant within 3 months before study entry (screening visit)

![Graph showing effect of daily intravaginal application of DHEA on percentage of vaginal parabasal cells in postmenopausal women.](image)

**FIG. 1.** Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the percentage of vaginal parabasal cells in postmenopausal women. Data are expressed as means ± SEM; the P values for the three DHEA doses are comparisons with placebo at all time intervals, whereas for the placebo group at 12 weeks, comparison is with baseline.
- Use of estrogen pellet or progestin injectable drug within 6 months before study entry (screening visit)
- Use of oral estrogen, progestin, or DHEA exposure or intrauterine progestin therapy in the 8 weeks before baseline assessments (screening visit)
- Use of vaginal hormonal products (rings, creams, or gels) or transdermal estrogen alone or estrogen/progestin products in the 4 weeks before baseline assessments (screening visit)

Participants could washout as follows, but the questionnaire on vaginal atrophy was answered after the required washout period.

- At least an 8-week washout period for prior oral estrogen, DHEA, and/or progestin therapy
- At least a 4-week washout period for prior transdermal hormone therapy
- At least a 4-week washout period for locally delivered hormone therapy for vaginal dryness (rings, creams, gels, or tablets)
- At least 6 months for prior estrogen pellet therapy or progestin injectable drug therapy
- Eight weeks or longer for prior intrauterine progestin therapy
- Six months or longer for prior progestin implants and estrogen-alone injectable drug therapy
- Previous treatment with androgens or anabolic steroids within 3 months before screening visit
- Oral corticosteroid treatment within 6 weeks of study start (day 1)
- No chronic use of corticosteroid allowed (intermittent nasal spray or topical on skin, eyes, or ears is permitted)
- Cardiac failure or manifest coronary heart disease
- Hypertension equal to or above 160/95 mm Hg or not controlled by standard therapy
-Confirmed clinically significant depression or confirmed history of severe psychiatric disturbance
- The administration of any investigational drug within 30 days of screening visit
- Clinically relevant abnormal serum biochemistry, hematology, or urinalysis
- Baseline cervical cytology showing low-grade squamous intraepithelial lesion or worse
- Smoking more than 10 cigarettes a day
- Drugs that interfere with the metabolism of estrogens (eg, ketoconazole), steroid formation, or action inhibitors
- Selective estrogen receptor modulators or drug interacting with steroid receptors
- Known presence of uterine fibroma or fibroma palpable at gynecological examination
- Coagulation disorders or receiving anticoagulant drug therapy

Informed consent

Written informed consent was obtained from all participants before the performance of any study-related procedure. The participants had a medical history, a medical examination, and a complete gynecological examination (including an endometrial biopsy) at screening. A partial gynecological examination was performed to evaluate the aspect of the mucosa and tolerance to the medication on day 1 and at weeks 2, 4, 8, and 12.

Laboratory tests

The standard laboratory tests, namely, hematology (including complete blood count and coagulation), blood chemistry, and urinalysis were performed at screening and at week 12 (Table 1). Serum follicle-stimulating hormone was measured only in women who had no menses for 6 months or more but less than 12 months or who were premenopausal at the time.
of hysterectomy. Serum steroid levels of DHEA; DHEA-sulfate; androst-5-ene-3β,17β-diol; dihydrotestosterone; testosterone; androstanediol; estrone (E₁); estradiol (E₂); E₁ sulfate (E₁-S); androsterone glucuronide; androstan-3α 17β-diol-3G; and 3α-diol-17G were measured at the Laboratory of Molecular Endocrinology, Laval University Hospital Centre Research Center, by mass spectrometry as described²⁶,²⁹,³²,³³,³⁵,³⁶ (data are presented in the accompanying article)³⁷.

Vaginal pH and cytology and endometrial histology

Endometrial histology was examined by Dr. Robert Dubé and Dr. Valérie Dubé (Department of Cytology-Pathology, Centre Hospitalier Affilié Universitaire de Québec, Quebec City, QC, Canada) and they were blinded to the treatment regimens. For the vaginal cell maturation evaluation, samples were examined by Lucie Genest and Claudette Girard, under the supervision of Dr. Robert Dubé while Pap tests were evaluated at the site’s local laboratories. A 100-cell count was performed to classify cells as superficial, intermediate, and parabasal squamous cell types.³⁸,³⁹ Vaginal smears were obtained by scraping the second third of the side wall of the vagina with the rounded end of an Ayre spatula. The material was then applied to a glass slide and immediately fixed with Spray-Cyte. These samples were sent to the central laboratory for determination of the maturation index. Vaginal pH was measured by applying a pH indicator strip directly to the lateral wall of the vagina with a forceps. For the Pap smear—if not done in the last 12 months—specimens were obtained from the endocervix, exocervix, and vaginal vault and immediately fixed with cytospray. The specimens were collected with an Ayre spatula.

Mammography

Mammography was performed if not done during the past 9 months.

Endometrial biopsy

Endometrial biopsy was performed at screening and at the end of the study (12 wk). All biopsies were examined by the same pathologist at the central laboratory (Dr. Robert Dubé).

Vaginal examination

Vaginal examination was performed at screening and then at day 1 and weeks 2, 4, 8, and 12. Vaginal secretions, vaginal color, vaginal epithelial integrity, and vaginal epithelial surface thickness were evaluated according to the following degrees of severity: none, mild, moderate, or severe. The definitions of severity were as follows:

a) Vaginal secretions

- No atrophy: normal clear secretions noted on vaginal walls
- Mild: superficial coating of secretions, difficulty with speculum insertion
- Moderate: scant not covering the entire vaginal vault, may need lubrication with speculum insertion to prevent pain
- Severe: none, inflamed, ulceration noted, need lubrication with speculum insertion to prevent pain

b) Vaginal epithelial integrity

- No atrophy: normal
- Mild: vaginal surface bleeds with scraping

FIG. 3. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the maturation value of vaginal epithelial cells in postmenopausal women. Values are expressed as means ± SEM; the P values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.
Moderate: vaginal surface bleeds with light contact
Severe: vaginal surface has petechiae before contact and bleeds with light contact
c) Vaginal epithelial surface thickness
No atrophy: rogation and elasticity of vault
Mild: poor rogation with some elasticity noted of vaginal vault
Moderate: smooth, some elasticity of vaginal vault
Severe: smooth, no elasticity, constriction of the upper one third of vagina or loss of vaginal tone (cystocele and rectocele)
d) Vaginal color
No atrophy: pink
Mild: lighter in color
Moderate: pale in color
Severe: transparent, either no color or inflamed

Statistics

Summary tabulations were prepared that display the number of observations, mean or geometric mean as appropriate, SD, SEM, 95% two-sided CI, median, minimum, and maximum for continuous variables, and the number and percent per category for categorical data. Statistical analyses were performed at the two-sided significance level of 0.05, unless otherwise stated. The categories for summarization generally consisted of the dose levels of the DHEA treatments: 0% (placebo), 0.25%, 0.5%, and 1.0% DHEA. The analysis comparing each dose of DHEA to placebo was performed by analysis of covariance, with the baseline value used as the covariate. The changes from baseline within each treatment group were assessed for statistical significance using paired t tests. Baseline was defined as the value on day 1, obtained before the first use of study treatment, and change from baseline to weeks 2, 4, 8, and 12 was calculated for each participant for each domain endpoint. Missing values for any endpoint for a participant were replaced using a last value carried forward approach.

The primary endpoints for analysis consisted of the following:
- Statistically significant decrease in parabasal cells and a statistically significant increase in superficial cells. The data were measured as a percentage of a total of 100 cells analyzed per smear. The maturation value was also calculated.
- Statistically significant lowering of vaginal pH
- Statistically significant improvement in the moderate to severe symptom identified by the participant as most bothersome to her. The symptom severity was based on symptoms of increasing severity: none, mild, moderate, or severe. These ratings were analyzed using the values 0, 1, 2, and 3, respectively; all participants needed to have at least one baseline symptom graded as 2 or 3. The symptoms of interest were vaginal dryness, vaginal and/or vulvar irritation/itching, and vaginal pain associated with sexual activity.

Analysis populations

The ITT population consisted of the treated participants with a baseline and at least one postbaseline efficacy assessment. Participants who may have received the wrong treatment were to be analyzed as randomized. This analysis population was considered the primary analysis population. Participants in this
population who had missing observations postbaseline had the last value carried forward for efficacy analyses.

The safety population was defined as all participants who received one administration of either test article (DHEA at any dose) or placebo and who had any safety information available. All safety data analyses were based on this population. Analysis was based on the treatment actually received.

**Efficacy evaluation**

Efficacy analyses were performed on the ITT population. The primary study objective was to evaluate the dose response of vaginal mucosal parameters to the local action of DHEA in postmenopausal women experiencing vaginal atrophy, specifically by determination of the minimal dose of DHEA that produces maximal effect on the vaginal mucosa. The coprimary efficacy endpoints to address this objective were decrease in parabasal cells, decrease in vaginal pH, increase in superficial cells, and improvement of participant self-reported most bothersome symptom among vaginal dryness, vaginal and/or vulvar itching/irritation, and vaginal pain associated with sexual activity. The self-reported symptom scores had the values, none, mild, moderate, or severe, and were analyzed using values of 0, 1, 2, or 3, respectively. All endpoints had to demonstrate statistically significant effects relative to placebo. The primary time point for analysis was the 12-week assessment, with additional presentations of the data for 2, 4, and 8 weeks.

**RESULTS**

Because parabasal cells are usually the predominant category in the vaginal smear of postmenopausal women with at least one moderate to severe symptom of vaginal atrophy, it can be seen in Fig. 1 that already at 2 weeks of treatment, the lowest dose of DHEA (0.25%) decreased the percentage of parabasal cells by 28.6 ± 4.95 from 56.9 ± 5.54 to 28.3 ± 4.34, whereas decreases of 37.1 ± 4.55 (from 58.8 ± 5.37 to 21.6 ± 3.47) and 36.0 ± 4.91 (from 48.9 ± 5.46 to 12.9 ± 2.81) were observed, respectively, with the 0.5% and 1.0% DHEA doses at the same early time interval of 2 weeks. At the standard duration of 12 weeks of treatment, decreases of 39.8 ± 5.33, 45.9 ± 5.31, and 44.5 ± 5.19 were observed in the percentage of parabasal cells with the 0.25%, 0.5%, and 1.0% DHEA doses, respectively, whereas no significant effect was observed in the placebo group at any time interval. It can also be seen in Fig. 1 that the effect of DHEA is rapid, with 72%, 81%, and 81% of the effect measured at 12 weeks being already observed at 2 weeks at

**TABLE 2. List of number of women reporting vaginal atrophy symptoms of various degrees of severity at baseline**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Dryness</th>
<th>Irritation/itching</th>
<th>Pain at sexual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None = 0</td>
<td>15</td>
<td>61</td>
<td>2</td>
</tr>
<tr>
<td>Mild = 1</td>
<td>35</td>
<td>85</td>
<td>27</td>
</tr>
<tr>
<td>Moderate = 2</td>
<td>118</td>
<td>57</td>
<td>49</td>
</tr>
<tr>
<td>Severe = 3</td>
<td>48</td>
<td>13</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>216</td>
<td>188*</td>
</tr>
</tbody>
</table>

*Twenty-eight women reported no sexual activity at baseline.

**TABLE 3. Most bothersome symptom identified by participants at baseline**

<table>
<thead>
<tr>
<th>Dryness</th>
<th>Irritation/itching</th>
<th>Pain at sexual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>66</td>
<td>16</td>
</tr>
</tbody>
</table>
TABLE 4. Change from day 1 (baseline) in the categories of severity scores of the most bothersome symptoms at 12 weeks of treatment with 0% (placebo), 0.25%, 0.50%, and 1.0% DHEA

<table>
<thead>
<tr>
<th>Doses</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>3.77</td>
<td>15.09</td>
<td>20.75</td>
<td>54.72</td>
<td>5.66</td>
</tr>
<tr>
<td>0.25%</td>
<td>7.84</td>
<td>35.29</td>
<td>25.49</td>
<td>29.41</td>
<td>1.96</td>
</tr>
<tr>
<td>0.5%</td>
<td>21.82</td>
<td>29.09</td>
<td>27.27</td>
<td>21.82</td>
<td>0.02</td>
</tr>
<tr>
<td>1.0%</td>
<td>11.54</td>
<td>34.62</td>
<td>30.77</td>
<td>21.15</td>
<td>1.92</td>
</tr>
</tbody>
</table>

*Change from one category (severe → moderate → mild → none) was taken as −1, whereas a change of two categories was −2, etc. Data are expressed as a percentage of the total of the symptoms for each DHEA dose.*

DHEA, dehydroepiandrosterone.

The above-indicated increasing DHEA doses. The effect of all doses of DHEA was highly significant from placebo at all time intervals (*P < 0.0001* for all).

Although no significant change was seen at 12 weeks in the placebo group in the percentage of superficial cells (Fig. 2), increases of 4.3 ± 0.96 (from 0.7 ± 0.26 to 5.0 ± 0.90, *P* = 0.009 vs placebo), 6.8 ± 1.29 (from 0.5 ± 0.16 to 7.2 ± 1.29, *P* < 0.0001), and 5.8 ± 1.13 (from 0.9 ± 0.24 to 6.7 ± 1.16, *P* = 0.0002) were measured in the 0.25%, 0.5%, and 1.0% DHEA groups, respectively. It can also be seen that with the 0.5% DHEA, 52% of the maximal effect was achieved at 2 weeks, whereas at 4 and 8 weeks, 85% and 99% of the maximal effect were observed. At the 1.0% DHEA dose, 93% of the maximal effect seen at 8 weeks was already reached at 2 weeks. All doses of DHEA had a highly significant effect above placebo (*P* = 0.009, *P* = 0.0002, and *P* < 0.0001) at 12 weeks, whereas at 4 and 8 weeks, the *P* values were both less than 0.0001 for the 0.5% and 1.0% doses and 0.016 and 0.019, respectively, for the 0.25% dose. At 2 weeks, the *P* values were 0.082, 0.017, and less than 0.0001 compared with placebo for the 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

Analysis was also made of the maturation value of the vaginal smear at the same time intervals. As can be seen in Fig. 3, the maturation value obtained by multiplying the percentage of parabasal cells by 0, the percentage of intermediate cells by 0.5, and the number of superficial cells by 1.038 went from 21.0 ± 2.72 on day 1 to 41.2 ± 1.84, 45.2 ± 1.67, 46.5 ± 1.51, and 47.3 ± 1.58 at 2, 4, 8, and 12 weeks, respectively, with the 0.5% DHEA dose. Comparable values were obtained with the 1.0% dose, whereas slightly smaller changes were observed with the 0.25% dose. It can be seen in Figure 3 that the difference between treatment and placebo was highly significant (*P < 0.0001*) at all doses and at all time intervals.

Vaginal pH was decreased at 12 weeks by 1.1 ± 0.12 units (*P < 0.0001*) from 6.5 ± 0.09 units, by 1.3 ± 0.13 units (*P < 0.0001*) from 6.6 ± 0.07 units, and by 1.4 ± 0.13 units (*P < 0.0001*) from 6.3 ± 0.09 units in the 0.25%, 0.50%, and 1.0% DHEA-treated groups, respectively (Fig. 4). In the placebo group, pH decreased by 0.4 ± 0.11 unit (*P* = 0.0002) at 12 weeks from 6.5 ± 0.09 units. At the 0.5% DHEA dose, 77%, 92%, and 100% of the maximal effect on pH (reduction of 1.3 pH units) was achieved at 2, 4, and 8 weeks of treatment, respectively. As indicated in Fig. 4, the difference between treatment and placebo is highly significant at all doses and at all time intervals (*P = 0.0008* to <0.0001).

At screening, all women needed to have one or more of the following symptoms of vaginal atrophy evaluated by herself as moderate to severe: vaginal dryness, vaginal or vulvar irritation/itching, or vaginal pain at sexual activity. The self-identified symptoms reported during the course of the study as none, mild, moderate, or severe were analyzed using values of 0, 1, 2, and 3, respectively. The women also needed to identify one of the three symptoms as being the most bothersome, which was a coprimary objective along with the changes in vaginal parabasal cells, superficial cells, and pH. Figure 5 illustrates the changes in the severity score of the most bothersome symptoms at the different DHEA doses and time intervals. As indicated in this figure, at the 12-week interval, the severity of the most bothersome symptom was reduced by 0.6 ± 0.13 in the placebo group, 1.5 ± 0.14 in the 0.25% DHEA group (*P* = 0.002 vs placebo), 1.5 ± 0.14 in the group receiving 0.5% DHEA (*P* < 0.0001 vs placebo), and 1.3 ± 0.14 in the group receiving the 1.0% DHEA dose (*P* = 0.0001 vs placebo).

Starting at 8 weeks for the 0.25% DHEA dose (*P* = 0.008), 4 weeks for the 0.5% dose (*P* = 0.03), and 2 weeks for the 1.0% dose (*P* = 0.02), the difference with placebo was significant to highly significant at all time intervals and doses (*P* = 0.03 to <0.0001). In the 0.25%, 0.5%, and 1.0% DHEA groups, the effect was clearly time dependent because only 50%, 53%, and 62% of the effect, respectively, observed at 12 weeks were achieved at 2 weeks. At 8 weeks, however, 75%, 73%, and 92% of the maximal change of the most bothersome symptom were observed at the three increasing DHEA doses.
As indicated in Table 2, the presence of vaginal dryness, irritation/itching, and pain at sexual activity of moderate to severe intensity was reported at baseline by 166, 70, and 159 women, respectively. Twenty-eight women did not report sexual activity at baseline evaluation (day 1). Only 16 women identified irritation/itching as the most bothersome symptom, whereas 66 and 129 women, respectively, identified dryness and pain at sexual activity as the most bothersome symptom (Table 3).

The percentage of women with no change or a worsening by a score of 1 of the most bothersome symptom at 12 weeks decreased from 60.4% in the placebo group to 31.4%, 21.8%, and 23.1% in the 0.25%, 0.5%, and 1.0% DHEA groups, respectively (Table 4). Improvement by two or three categories of the severity score was observed in 18.9% of women treated with placebo, whereas 43.1%, 50.9%, and 46.2% of women who received the 0.25%, 0.5%, and 1.0% DHEA formulations reported such an improvement. Only 3.8% of women indicated a decrease from severe to none in the placebo group compared with 7.8%, 21.8%, and 11.5% in the DHEA-treated groups.

As illustrated in Fig. 6, vaginal pain at sexual activity, the symptom identified as being the most bothersome by most women, remained unchanged in 63.3% of women in the placebo group, whereas it decreased to 29.0%, 26.7%, and 19.3% in the groups of women receiving the 0.25%, 0.5%, and 1.0% DHEA doses, respectively (Table 5). Improvement by two or three categories of the severity score was observed in 18.9% of women treated with placebo, whereas 43.1%, 50.9%, and 46.2% of women who received the 0.25%, 0.5%, and 1.0% DHEA formulations reported such an improvement. Only 3.8% of women indicated a decrease from severe to none in the placebo group compared with 7.8%, 21.8%, and 11.5% in the DHEA-treated groups.

As illustrated in Fig. 6, vaginal pain at sexual activity, the symptom identified as being the most bothersome by most women, remained unchanged in 63.3% of women in the placebo group, whereas it decreased to 29.0%, 26.7%, and 19.3% in the groups of women receiving the 0.25%, 0.5%, and 1.0% DHEA groups, respectively.
and 1.0% DHEA doses, respectively. The effect of DHEA treatment was particularly well illustrated by a decrease of three scores (severe [3] to none) in 0.0%, 9.7%, 30.0%, and 12.9% of women treated with the 0.0%, 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

It is of interest that although the beneficial effect of placebo on vaginal pain was observed in 30% of women who had this symptom identified as the most bothersome at the start of study, a much greater effect of placebo was found on vaginal dryness, namely, in 62.5% of women who had this symptom as most bothersome (Table 5). Despite this large placebo effect on such a highly subjective parameter, vaginal dryness improved in 78% and 80% of women who received the 0.5% and 1.0% DHEA doses, respectively.

At 12 weeks, the standard time interval, the severity score of vaginal dryness identified at screening at any level of severity was decreased by 47%, 53% (NS vs placebo), 63% \( (P = 0.014 \text{ vs placebo}) \), and 68% \( (P = 0.014 \text{ vs placebo}) \) at the 0%, 0.25%, 0.5%, and 1.0% DHEA doses (Fig. 7). For irritation/itching identified as the most bothersome symptom by only 16 women, a statistically significant difference from placebo was observed with the 0.5% dose at 8 weeks \( (P = 0.05 \text{ vs placebo}) \) and 12 weeks \( (P = 0.002 \text{ vs placebo}) \) (data not shown).

The most important effect of DHEA among the vaginal atrophy symptoms was observed on pain at sexual activity, the most bothersome symptom identified by most women \( (129/216) \) (Table 3). As can be seen in Fig. 8, at 12 weeks, the 0.25%, 0.5%, and 1.0% doses of DHEA caused 50% \( (P = 0.0009 \text{ vs placebo}) \), 60% \( (P < 0.0001 \text{ vs placebo}) \), and 58% \( (P < 0.0001 \text{ vs placebo}) \) decreases in the severity of this symptom. The effect was already significant at 8, 8, and 2 weeks for the 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

At the same time intervals of 2, 4, 8, and 12 weeks, the gynecologist or physician in charge of the clinical trial at each study site performed a vaginal examination to evaluate the severity (none, mild, moderate, or severe) of vaginal dryness, namely, vaginal secretions, vaginal color, vaginal epithelial integrity, and vaginal epithelial surface thickness. As can be seen in Figs. 8 to 12, a time-dependent and dose-related as well as highly statistically significant improvement of all four signs of vaginal atrophy was seen. In fact, the beneficial effects observed by the gynecologist or physician in charge are almost superimposable to those self-reported by women on their most bothersome symptoms as well as to the effects on vaginal parabasal and superficial cells and pH, the coprimary objectives of DHEA action described above.

As shown in Fig. 9, a rapid effect of treatment is observed on vaginal secretions as early as 2 weeks with the three doses of DHEA administered intravaginally, the difference with placebo being highly significant versus placebo at all doses of DHEA and durations of treatment, the effect increasing with the duration of treatment and with dose. At 12 weeks, the severity score decreased by 39%, 44%, and 47% in the 0.25%, 0.5%, and 1.0% DHEA groups (all \( P < 0.0001 \text{ vs placebo} \) (Fig. 9). For vaginal color (Fig. 10), similar findings were observed with highly significant differences with placebo \( (P = 0.008 \text{ to } P < 0.0001) \) at 12 weeks at all doses. At
12 weeks, 33%, 42%, and 48% improvements in the color score were observed ($P = 0.0002$ vs placebo [0.25% DHEA]) or $P < 0.0001$ vs placebo [0.5% and 1% DHEA]).

When the epithelial integrity score was considered, 37%, 46%, and 48% improvements were observed at 12 weeks for the 0.25%, 0.5%, and 1.0% DHEA doses (all $P < 0.0001$ vs placebo; Fig. 11). For the epithelial surface thickness, on the other hand (Fig. 12), similar improvements of 34%, 42%, and 47% were observed at 12 weeks for the three increasing doses of DHEA (all $P < 0.0001$ vs placebo).

Concerning safety, no drug-related significant adverse event was observed in the present study nor in our previous
1-week pharmacokinetics study with doses of 0.5%, 1.0%, and 1.8% DHEA ovules.\textsuperscript{32,33} No adverse effect was observed on hepatic tests, hematocrit, or any hematological or biochemical parameter. Moreover, no significant adverse event related to DHEA was observed in a study performed in 75 women randomized to receive 3 g of 0.3% DHEA percutaneously twice daily for 12 months\textsuperscript{35} or 15 women who received daily 3 to 5 g of 10% DHEA cream for 1 year.\textsuperscript{34}

**DISCUSSION**

The present data show for the first time that the local administration of DHEA, the essential precursor of all sex
steroids but an inactive compound by itself, is highly efficient and rapid in correcting all the symptoms and signs of vaginal atrophy in postmenopausal women. Most importantly, this is achieved at DHEA doses, which have no or minimal effects on serum estrogens and androgens and their metabolites, which all remain well within the range of values found in normal postmenopausal women. Despite their well-recognized safety issues, various forms of estrogens are an efficient treatment for vulvovaginal atrophy. In fact, the vaginal E₂ tablet has shown an efficacy similar to that of the E₂ ring and the conjugated estrogen cream. It is estimated, however, that approximately 40% of women taking oral hormone therapy have persistent vaginal dryness.

As mentioned above, although 75% of postmenopausal women suffer from vaginal atrophy, thus affecting their quality of life during a major part of their lifetime, only 20% seek treatment. The fear of breast cancer associated with the prescription of estrogens which increases the serum levels of estrogens is the main reason for the lack of acceptance of estrogen therapy by most women and their physicians. In the aftermath of Women’s Health Initiative study, the scientific challenge is to explore alternative hormone therapy types and formulations that would provide all the menopausal advantages of estrogens while improving women’s quality of life, minimizing risks, and maximizing benefits.

It is pertinent to mention that because estrogen secretion into the systemic circulation in women ceases at menopause, administration of estrogens to postmenopausal women is not a physiological solution. On the other hand, because the nonestrogen-based treatments have not shown convincing efficacy, women and their physicians are left with no safe treatment for vaginal atrophy.

A recent study in which serum steroid levels were measured at multiple time intervals to obtain maximal precision and information during the 24-hour period has shown that after daily treatment with 0.5% or 1.0% DHEA ovules, only serum DHEA and androst-5-ene-3β,17β-diol (and androstenedione at day 1 and E₁ on day 7) are increased significantly from baseline but well within the limits of values found in normal postmenopausal women. Serum estrogens (E₂ and E₁-S) and serum androgens (testosterone and dihydrotestosterone) are not significantly affected. Although DHEA can be transformed into both androgens and estrogens in the mammary gland, the global effect of DHEA on the human mammary gland is inhibitory. Most importantly, the metabolites of androgens (androstosterone glucuronide; androstan-3α,17β-diol-3G, and 3α-diol-17G) and estrogens (E₁-S) are unchanged or minimally changed after intravaginal DHEA administration, thus clearly demonstrating the absence of significant systemic androgenic and estrogenic formation and, therefore, potential systemic effects of sex steroids at the doses used.

In addition to the increased breast cancer risk associated with the administration of estrogens, it is important to recognize that the specific hormonal difference between the postmenopausal women who do not have vaginal atrophy (estimated at 25% of the postmenopausal population) and the majority (75%) of postmenopausal women who have vaginal atrophy is absolutely not related to the secretion of estrogens in the systemic circulation because estrogen secretion by the ovaries has ceased in all women at time of menopause. Consequently, a deficit in estrogen secretion into the systemic circulation is not a valid explanation for the occurrence of symptoms of vaginal atrophy in 75% of postmenopausal women, whereas the remaining and more fortunate 25% of women live throughout all their postmenopausal years without significant bothersome or evident vaginal atrophy symptoms. Therefore, estrogens may not be a physiological replacement therapy for the 75% of postmenopausal women who have vaginal atrophy, as other factors may be at play.

Contrary to the estrogens of ovarian origin, which are secreted into the general circulation where they are exposed to all tissues, DHEA is an inactive precursor that is transformed only in the peripheral tissues that possess the required steroidogenic enzymes. The process of intracrinology thus permits local intratissular formation of active sex steroids with no significant release of the active steroids into the circulation, thus avoiding exposure of the other tissues not physiologically in need of these active sex steroids.

As mentioned above, however, the secretion of DHEA, the only source of sex steroids after menopause, markedly and variably decreases with age, with an average 60% loss being already observed at time of menopause. Consequently, the only difference between the symptomatic (75%) and the asymptomatic (25%) postmenopausal women is the amount of DHEA secreted by the adrenal glands, the ability of each tissue to transform DHEA into active sex steroids, and/or the sensitivity of the vaginal tissue to DHEA. The difference in sensitivity of different women is likely to be related, up to an unknown extent, to the level of activity of the enzymatic machinery specific to each cell type in each tissue. With this knowledge, it is clear that DHEA and not estrogens should be the physiological hormone therapy at menopause.

Change in pH is now recognized as a valid parameter that reflects the beneficial effect of therapy on vaginal atrophy. After 12 weeks of intravaginal treatment with 25 μg E₂, the percentage of participants having a pH less than 5.0 was 51% compared with 21% in the placebo group. At baseline, however, 11.2% and 13% of women in that study already had a pH below 5.0 in the two corresponding groups. In the present study, no participant had a pH value below 5.0 at screening, whereas 29%, 53%, 61%, and 71% had pH values at or below 5.0 at 12 weeks in the 0%, 0.25%, 0.5%, and 1.0% DHEA groups, respectively.

As demonstrated in the present study, the effect of DHEA on the maturation of the vaginal epithelial cells is particularly rapid: with the 0.5% DHEA ovule, 81% of the maximal effect on parabasal cells was already observed at 2 weeks, whereas 52% of the maximal stimulatory effect exerted on
superficial cells was observed at the same time interval. On
the other hand, 85% of the maximal effect of 0.5% DHEA
on the percentage of superficial cells was achieved at 4
weeks. Similarly, 53% of the maximal effect of 0.5% DHEA
on the most bothersome symptom was observed at 2 weeks,
and 73% was reached at 4 weeks. Moreover, only 17.8% of
women reported no improvement in their most bothersome
symptom at 12 weeks in the 0.5% DHEA group compared
with 48.8% in the placebo group.

The effect of DHEA on parabasal cells is especially rapid
because the percentage of parabasal cells was decreased to
less than 20% at 1 month with the three DHEA doses used.
The effect on the percentage of superficial cells is also very
rapid, with 96% of the effect being seen at 2 weeks with the
high (1%) DHEA dose. In a study with vaginal estrogen
cream or tablet, approximately 50% of the effect measured at
12 weeks was observed at 2 weeks. Such data indicate that
the rapidity of the effect of DHEA is not inferior and is
possibly superior to the effect of the vaginal E₂ and
conjugated estrogen formulations.

In a recent study, the vaginal maturation value increased
from 27.4 at baseline to 56.8 (P < 0.0001) in the estrogen-
treated group. The percentage of superficial cells increased
by 17.1 from baseline, whereas the percentage of parabasal
cells decreased by 41.7% in the estrogen-treated group. In the
same study, the vaginal pH decreased from 6.74 at baseline
to 5.05 (decrease of 1.69% or 24% in the estrogen group).
The severity of the most bothersome symptoms decreased from
2.58 to 1.04 (−1.54) in the estrogen group compared with
a decrease from 2.59 to 1.84 (−0.75) in the placebo
group. Such data observed with estrogens are comparable to
the 1.56 decrease in severity of the most bothersome
symptoms at 12 weeks in the 0.5% DHEA group and the
0.67 decrease in the placebo group observed in our study.

At week 12, 11% of Estrin participants and 24% of
Vagifem participants had persistent atrophic epithelium. At
week 48, the respective values were 8% and 14%. At 48
weeks of treatment with Vagifem or Estrin, vaginal dryness
was still present in 33% of women. Pruritus vulvae, on the
other hand, remained present in 15% and 20% of women
after treatment with Estrin and Vagifem, respectively,
whereas 33% and 28% of women still had dyspareunia after
treatment with Estrin and Vagifem, respectively. Bleding
after the progestogen test was 7% in the Vagifem group and
0% in the Estrin group. In the present study, after 12 weeks
of treatment with the 0.25%, 0.5%, and 1.0% DHEA doses,
improvement of the most bothersome symptom was observed
in 72.5%, 82.4%, and 81.4% of women, respectively, thus
leaving only 23.5%, 17.8%, and 19.6% of women with no
reported improvement of their most bothersome symptoms.

After 3 months of daily administration of 0.625 mg
Premarin orally or intravaginally (cream), respective 70.6%
and 75% improvements of dyspareunia were observed. It
was concluded in that study that 1 g of 0.625 mg Premarin
was the minimal dose for the treatment of sexual dysfunction.
In women who received 25 μg E₂ intravaginally, dyspareunia
persisted in 12.4% after 12 months of treatment. The
success rate of therapy of local E₂ tablets was 84.5% as
judged by participants and 86.1% as judged by physicians,
but 40% to 50% of women receiving oral estrogen therapy
had persistent complaints of vaginal dryness.

As reported previously after 12 months of treatment with
DHEA, the present study shows no effect on endometrial
histology after 3 months of intravaginal administration of the
hormone precursor DHEA as shown by histopathological
examination of the endometrial biopsies obtained before and
after 12 weeks of treatment. These findings are in agreement
with the absence of aromatase activity in the human
endometrium. This observation is also strongly supported
by the well-recognized clinical observation that endometrial
atrophy is characteristic of postmenopause despite the
continuous secretion of DHEA. The absence in the
human endometrium of the steroidogenic enzymes necessary
to transform DHEA into estrogens is in agreement with the
physiological role of the endometrium, which is active
exclusively during the reproductive years when its function
is essentially controlled by hormones of ovarian and placental
origins. In fact, there is no physiological role of the endo-
metrium after menopause which would justify any need for
estrogens. Moreover, exposure of the postmenopausal endo-
metrium to estrogens is associated with a well-known in-
creased risk of endometrial cancer. Accordingly, the enzymes
required for the synthesis of estrogens from DHEA are not
expressed in the endometrium, a tissue fully dependent on
estrogens of ovarian origin and limited in its function to pre-
menopause and pregnancy.

We believe that the androgenic component of DHEA
action plays a so-far unrecognized role in general vaginal
health. In fact, as mentioned above, our recent preclinical
data have shown an important effect of DHEA on all three
layers of the vaginal wall, including the collagen fibers of the
lamina propria and the muscularis. It is expected that such
a global action of DHEA exerts benefits on the severity of the
signs of vaginal atrophy, especially those related to a reduc-
tion in the length and diameter of the vagina, the loss of va-
ginal rugal folds, and the disappearance of vaginal fornices.
All these atrophic changes are accompanied by decreased
vascularization, decreased vaginal secretions, and more sus-
ceptibility to trauma and pain. The specific androgenic effect
on collagen formation could play a major role in the parti-
cularly positive results obtained in this study.

CONCLUSIONS

The Food and Drug Administration Guidance to Industry
encourages sponsors to develop the lowest doses and ex-
posures for both estrogens and progestins. We must recog-
nize that although estrogens are efficient in correcting the
symptoms of vaginal atrophy and vasomotor symptoms, sys-
temic estrogens are not the physiological hormones that
permit reduction of symptoms of vaginal atrophy in ap-
proximately 25% of postmenopausal women who remain
relatively asymptomatic throughout their postmenopausal years.2 Because the only variable source of sex steroids available in postmenopausal women is DHEA secreted by the adrenals and locally transformed in specific cells and tissues into estrogens and/or androgens by the mechanisms of intracrinology, 44,47 replacement with DHEA seems to be the only physiological replacement therapy for postmenopausal women experiencing menopausal symptoms. With the novel approach that we have called tissue-specific prehormone replacement therapy, we theorize vaginal atrophy symptoms should be corrected with no more risk than those present in the 25% of postmenopausal women who have no symptoms of vaginal atrophy because of the presence in these women of a higher exposure or sensitivity to DHEA.

REFERENCES