Intravaginal prasterone (DHEA) provides local action without clinically significant changes in serum concentrations of estrogens or androgens

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A B S T R A C T

In order to avoid the risks of non-physiological systemic exposure, serum concentrations of estradiol (E2) and testosterone (as measured by mass spectrometry-based assays) should remain below the 95th centiles measured at 9.3 pg/ml and 0.26 ng/ml for these respective sex steroids in normal postmenopausal women. To document the possibility of achieving this therapeutic objective, we have measured individual 24 h serum E2 and testosterone concentrations in women with vulvovaginal atrophy (VVA) receiving daily intravaginal administration of a clinically effective dose of 6.5 mg prasterone (dehydroepiandrosterone, DHEA).

Serum E2 and testosterone, as well as DHEA and nine of its other metabolites, were assayed at ten time intervals over 24 h on the first and seventh days of daily vaginal administration of 6.5 mg prasterone.

No significant change from baseline of average 24 h serum E2 or testosterone concentrations was observed. Moreover, average 24 h serum DHEA remained within the normal postmenopausal range. Estrone sulfate and the androgen metabolites androstanol glucuronide and androstan-3-ol were not significantly observed.

Serum concentrations of metabolites of both estrogens and androgens remain within the normal postmenopausal range following daily intravaginal administration of 6.5 mg prasterone. As other studies have shown, local formation of sex steroids in peripheral tissues without significant release of E2 or testosterone in the circulation can be achieved with intravaginal prasterone. Thus, prasterone is a promising physiological and attractive solution to treating VVA symptoms.

1. Introduction

At time of menopause, under physiological conditions, secretion of estrogens in the circulation ceases [1]. Vulvovaginal atrophy (VVA) is a common and persistent condition with high prevalence, as approximately half of all postmenopausal women will experience symptoms related to urogenital atrophy [2,3].

Despite its established prevalence, only about 25% of symptomatic women seek medical help, partially due to reluctance to discuss intimate and private issues related to vaginal health [3].

Other than over-the-counter lubricants and moisturizers having limited efficacy, current treatment for VVA is limited to estrogen therapy [4]. Moisturizers and lubricants can provide temporary symptomatic relief (coital comfort), but they do not treat the underlying cause of the condition [5]. In addition, the majority of women with VVA are not treated and/or are looking for an alternative treatment to estrogen-based therapy.

The challenge for an effective and well tolerated treatment of the vaginal symptoms related to sex steroid deficiency following menopause is to avoid systemic exposure to estradiol (E2) and testosterone. An attractive approach to achieve this goal is through dehydroepiandrosterone (DHEA). Dehydroepiandrosterone, DHEA, an endogenous inactive compound by itself, is converted to estrogens and/or androgens in peripheral tissues which possess the required steroidogenic enzymes [6,7] into cell-specific intracellular E2 and testosterone by the mechanisms of intracrinology [8–10].
DHEA, especially in the brain, has been suggested to act through a series of neuronal signaling pathways [11].

Prasterone (DHEA), administered locally in the vagina, is a non-estrogen precursor that enters vaginal cells and gets converted intracellularly to both estrogens and/or androgens depending upon the cell type, thus exerting rapid beneficial effects on VVA [12] as well as on sexual dysfunction [13]. Outside the vaginal cell, there is no meaningful increase in estrogen (serum E2) or androgen (serum testosterone) concentrations [8–10].

Here, we detail the results of 24-h individual serum concentrations of E2, testosterone plus DHEA and nine other metabolites (from [9,10]) after the first and seventh daily administration of 0.5% (6.5 mg) intravaginal prasterone, the maximal dose used in current Phase III clinical trials for VVA.

2. Subjects and methods

2.1. Subjects

Forty postmenopausal women with one or more self-identified symptoms of VVA (criteria detailed in [9,10]) were included in this study. The mean age of the women who received the daily
intravaginal administration of 6.5 mg DHEA was 61 years (range, 53–69 years).

2.2. Study design and population

This was a randomized, double-blind study of 10 subjects per arm (40 subjects total) conducted at the Centre Hospitalier de l’Université Laval, Quebec, Canada.

The 40 postmenopausal women were randomized to receive a daily dose of one intravaginal ovule of the following prasterone (DHEA) concentrations: 0.0%, 0.5% (6.5 mg prasterone), 1.0% (13 mg prasterone), or 1.8% (23.4 mg prasterone). Blood samples were taken at 0 (time 0), 0.5, 1, 2.4, 6, 8, 12, 18, and 24 h following ovule administration [9]. Blood samples (17 ml/timepoint) were collected at the indicated intervals and approximately 5 ml (per timepoint) of serum was obtained following blood centrifugation at 4 °C for 10 min at 2400 × g. Data are shown here only for the 6.5 mg dose of prasterone, the maximal dose used in current Phase III clinical trials on VVA.

The study was divided into two phases, a screening period and a treatment period of 1 week. The protocol was approved by the Institutional Review Board of the Centre Hospitalier de l’Université Laval. Inclusion and exclusion criteria (plus results of serum DHEA and metabolite concentrations over 24 h) are as described in Ref. [9].

2.3. Measurement of steroids

Serum steroid levels of DHEA, DHEA-S, androstan-5-ene-3β, 17β-diol (5-diol), dihydrotestosterone (DHT), testosterone, androstenedione (4-dione), E₂, E₁, E₁-sulfate (E₁-S), androsterone glucuronide (ADT-G), androstane-3α, 17β-diol-3G (3α-diol-3G), and 3α-diol-17G were measured by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS) as described previously [9,14,15]. See Table 1 in Ref. [9] for the lower limits of quantification and within and between runs coefficients of variation. Deuterated compounds were used as internal standards [9].

Group mean (± standard error of the mean (SEM) or standard deviation (SD)) and individual 24 h steroid concentrations (areas under the 0–24 h curve) are reported from the first and seventh days of daily vaginal administration of 6.5 mg prasterone. Individual serum concentrations over 24 h post dosing (following the first and seventh days of daily vaginal administration of 6.5 mg prasterone) are reported for testosterone, DHT, ADT-G, DHEA, and DHEA-S.

2.4. Statistical analysis

Pharmacokinetic parameters were calculated on day 1 and day 7, including the area under the curve (AUC) from 0 to 24 h (AUC0–24h). AUCs were calculated by a linear trapezoidal method (model-independent). AUC0–24h and steroid concentrations were summarized using means and SEM or SD. Confidence intervals (95% two-tailed) were calculated for basal and average serum steroid levels. Statistical analysis of the day 7–day 1 differences was performed using paired t-tests within each group. Paired t-tests were used without adjustment for multiple comparisons, since this is a phase 1 study.

Table 1

<table>
<thead>
<tr>
<th>Serum steroid</th>
<th>Mean 24 h serum concentration (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d – 1</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>3.13 (0.37)</td>
</tr>
<tr>
<td>E₁ (pg/ml)</td>
<td>11.83 (1.28)</td>
</tr>
<tr>
<td>E₁-S (ng/ml)</td>
<td>0.13 (0.03)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>DHT (ng/ml)</td>
<td>0.026 (0.003)</td>
</tr>
<tr>
<td>ADT-G (ng/ml)</td>
<td>13.65 (3.71)</td>
</tr>
<tr>
<td>DHEA (ng/ml)</td>
<td>1.09 (0.24)</td>
</tr>
<tr>
<td>DHEA-S (μg/ml)</td>
<td>0.54 (0.16)</td>
</tr>
</tbody>
</table>

SEM, standard error of the mean; E₂, estradiol; E₁, estrone; E₁-S, E₁ sulphate; DHT, dihydrotestosterone; ADT-G, androsterone glucuronide; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulphate.
3. Results

3.1. Serum steroid concentrations

Following intravaginal administration of 6.5 mg prasterone, the 24h mean (±SEM) serum E2 concentrations (C_{avg} E2) showed no significant change from baseline with values of 3.13 ± 0.37 pg/ml, 3.66 ± 0.47 pg/ml (N.S. vs. baseline), and 4.04 ± 0.69 pg/ml (N.S. vs. baseline) on days -1 (baseline), 1 (first treatment day), and 7 (seventh treatment day) (Table 1). All these values are below the mean (±SD) serum E2 concentration reported as 4.17 ± 3.29 pg/ml in normal intact postmenopausal women aged 55–65 years of age [9]. Moreover, all of the individual 24h average E2 concentrations were below the 95th centile of 9.27 pg/ml observed in normal intact postmenopausal women [9]. Accordingly, mean 24h serum E2 concentrations did not differ from baseline on days 1 and 7 following daily vaginal administration of 6.5 mg prasterone, and no biologically significant change was observed, all values remaining within the normal postmenopausal range (Fig. 1).

After daily vaginal administration of 6.5 mg prasterone, mean (±SEM) 24h serum estrone (E1) concentrations on days -1 (baseline), 1, and 7 were 11.83 ± 1.28 pg/ml, 14.02 ± 1.58 pg/ml (N.S. vs. baseline), and 15.40 ± 2.04 pg/ml (N.S. vs. baseline) (Table 1 and Fig. 2). As observed for E2, all these values are below the mean (±SD) 24h serum E1 concentration in normal postmenopausal women reported as 17.78 ± 10.04 pg/ml [9]. It can also be seen in Fig. 2 that all individual average 24h E1 values were well below the 95th centile of normal postmenopausal women measured at 34.77 pg/ml [9].

In agreement with the absence of change in serum E2 and E1 following daily treatment with 6.5 mg prasterone (Table 1, Figs. 1 and 2), the average (±SEM) 24h serum E1-S levels showed no change following intravaginal prasterone (Table 1 and Fig. 3). In fact, serum E1-S levels were found to be 0.13 ± 0.03 ng/ml, 0.13 ± 0.02 ng/ml (N.S. vs. baseline), and 0.13 ± 0.03 ng/ml (N.S. vs. baseline) on days -1, 1, and 7 of treatment (Table 1). Moreover, individual average values following intravaginal prasterone were all within the normal postmenopausal range having a 95th centile at 0.59 ng/ml (Fig. 3).
Fig. 5. Serum dihydrotestosterone concentrations over a 24-h period. This figure displays serum levels of dihydrotestosterone (DHT) measured over a 24-h period in ten postmenopausal women following first (A) and seventh (B) daily vaginal administration of 6.5 mg prasterone. Individual and mean 24 h concentrations are indicated. For comparison and reference, steroid concentrations measured in 30–35-year-old premenopausal (n = 47) and 55–65-year-old postmenopausal (n = 377) women are also indicated [9,10].

The average (±SEM) 24 h serum testosterone concentration was 0.09 ± 0.01 ng/ml, 0.12 ± 0.01 ng/ml (N.S. vs. baseline), and 0.12 ± 0.01 ng/ml (N.S. vs. baseline) on days −1, 1, and 7, respectively (Table 1 and Fig. 4). These levels are below reported mean (±SD) serum testosterone levels (0.14 ± 0.07 ng/ml) in normal postmenopausal women [9]. Comparable results were observed with the low concentrations of serum DHT (Fig. 5). Baseline average serum DHT (on day −1) was 0.026 ± 0.003 ng/ml while on days 1 and 7, average serum DHT values over 24 h were 0.038 ± 0.004 ng/ml and 0.039 ± 0.004 ng/ml, respectively (Table 1), compared with the mean (±SD) of 0.04 ± 0.03 ng/ml in normal postmenopausal women [9]. On both days 1 and 7, serum DHT was below the limit of quantification (LLOQ) in some subjects. In those cases, the LLOQ value (0.02 ng/ml) was used in the mean calculation.

Concentrations of serum ADT-G (androsterone glucuronide, which represents approximately 93% of the sum of the androgen metabolite derivatives ADT-G, androstane-3α, 17β-diol-3G [3α-diol-3G], and 3α-diol-17G [14]) were unchanged on days 1 and 7 following daily intravaginal administration of 6.5 mg prasterone (Table 1 and Fig. 6). Subject S-213-007 had higher serum ADT-G values before starting treatment at 42.2 ng/ml at baseline. The 24 h average serum ADT-G values of this subject measured at 58.2 ng/ml and 47.13 ng/ml on days 1 and 7, respectively, remained well within the normal values of premenopausal women showing a 95th centile at 118.2 ng/ml [9].

The average 24 h serum DHEA concentrations remained below the postmenopausal 95th centile except for subject S-213-002 who had a higher value at 12 h on day 1 and at 6 h on day 7, and subject S-213-035 who showed higher values between 2 and 8 h after dosing on day 7 (Fig. 7). However, all average values over the 24 h period on days 1 and 7 were well within the normal postmenopausal range, with only one subject on day 1 and two women on day 7 having average values slightly above the 95th centile of normal postmenopausal women but well within the normal premenopausal range.

Serum DHEA-S concentrations were unchanged on days 1 or 7 compared with baseline (Table 1 and Fig. 8). Two subjects
Fig. 6. Serum androsterone glucuronide concentrations over a 24-h period. This figure displays serum levels of androsterone glucuronide (ADT-G) measured over a 24-h period in ten postmenopausal women following first (A) and seventh (B) daily vaginal administration of a 6.5 mg prasterone ovule. Individual and mean 24 h concentrations are indicated. For comparison and reference, steroid concentrations measured in 30–35-year-old premenopausal (n = 47) and 55–65-year-old postmenopausal (n = 377) women are also indicated [9,10].

(S-213-002 and S-213-035: the same two women who had somewhat higher serum DHEA concentrations; Fig. 7) had values close to the 95th centile seen in normal postmenopausal women before starting treatment and these values did not change following administration of intravaginal DHEA (Fig. 8). The average 24 h serum DHEA-S concentrations were measured at 0.54 ± 0.16 μg/ml, 0.57 ± 0.14 μg/ml (N.S. vs. baseline), and 0.55 ± 0.13 μg/ml (N.S. vs. baseline) at baseline, (day − 1) and day 7, respectively (Table 1).

The results for serum androst-5-ene-3β, 17β-diol (5-diol) were comparable with those observed for serum DHEA and with the same subject (S-213-002) showing a higher value at 4 h on day 1 and subject S-213-035 having higher values between 2 and 8 h after administration of intravaginal DHEA on day 7 (data not shown).

The average 24 h serum concentrations for androstenedione (4-dione) (the direct transformation product of DHEA by 3β-hydroxysteroid dehydrogenase) were well within the normal postmenopausal range with values (mean ± SEM) of 0.38 ± 0.04 ng/ml and 0.36 ± 0.03 ng/ml on days 1 and 7, respectively, compared with 0.40 ± 0.18 ng/ml (mean ± SD) in 55–65-year-old postmenopausal women (data not shown).

4. Discussion

The present data using low dose (6.5 mg) intravaginal prasterone (which exerts highly beneficial effects on VVA [12]) show that the average 24 h serum DHEA and all of the other nine metabolite concentrations remained within the normal postmenopausal range [8–10].

Estrogen-based formulations are currently the most effective therapy for VVA [16]. Generally, local estrogen administration is recommended when the therapeutic need applies only to symptoms of vaginal atrophy [17–19]. However, despite the lower systemic exposure of intravaginal compared with oral or percutaneous estrogens, serum E2 levels are increased above postmenopausal values (the 95th centile being measured at 9.3 pg/ml [9]) with intravaginal E2, even at the lowest dose currently...
available, whether measured by GC/MS (as reported here) or radioimmunoassay [21,22].

Mean serum E2 concentrations increased from approximately 40 pmol/l to a peak at about 90 pmol/l 8 h after first intravaginal administration of 10 μg E2, while it increased to about 150 pmol/l after administration of 25 μg E2 [21]. A smaller but significant increase in serum E2 was also seen on day 14 of daily administration of 10 μg E2 in the same study. A more recent placebo-controlled study (using one tablet daily for 14 days followed by one tablet twice a week, up to 52 weeks) has confirmed the beneficial effects of the 10 μg intravaginal E2 tablet [23], although a significant increase in serum E2 is observed at the low dosing regimen with a decrease in efficacy compared to higher estrogen doses [24,25].

VVA, unlike hot flushes, is a chronic, often progressive condition which does not tend to diminish over time and symptoms frequently recur following cessation of therapy [26]. Therefore, as long-term treatment of this condition is necessary, the avoidance of therapies which increase serum E2 concentrations is attractive. Thus, the challenge for a safe treatment of menopause is to keep systemic concentrations of E2 and testosterone within the postmenopausal range up to the 95th centile of 9.3 pg/ml and 260 pg/ml for these sex steroids, respectively [9]. No negative side-effects have been seen with physiological doses of exogenous DHEA, which is metabolized and acts identically to endogenous DHEA [27]. Thus, it is reasonable to believe that there should be no difference, with respect to sex steroid activity to avoid the symptoms of menopause, between those women who receive physiological exogenous DHEA doses to correct a clinically significant deficiency in DHEA and those with sufficiently high existing DHEA levels [27]. As suggested by Eugster-Hausmann et al., ‘in the absence of sufficient evidence specifically on the long-term overall safety of local estrogens, the amount of systemic absorption may be considered a surrogate endpoint, i.e., the less the increase in estrogen levels, the less potentially clinically relevant side-effects would be expected’ [20]. Appropriate prospective, randomized and placebo-controlled clinical trials similar to those performed for vaginal atrophy [8,12,13] are needed before therapeutic use of DHEA for other postmenopausal problems.
(e.g., bone and muscle loss) related to sex steroid deficiency can be recommended [27].

Conflict of interest

All authors are employees of EndoCeutics Inc. or received payment (from EndoCeutics Inc.) for their work.

Role of funding source

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References


