Transdermal estradiol and oral or vaginal natural progesterone: bleeding patterns

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ABSTRACT

Objective To evaluate the effects on bleeding pattern of two different doses of natural progesterone (NP) administered per os or per vagina in association with transdermal estradiol in a continuous, sequential estrogen–progestin therapy.

Methods A prospective, randomized trial was conducted on 100 patients randomized into four groups. Each group received transdermal 17β-estradiol treatment at the dose of 50 μg/day. Groups A and B received NP per os at the dose of 100 mg/day and 200 mg/day, respectively. Groups C and D received NP per vagina at the dose of 100 mg/day and 200 mg/day, respectively.

Results After 12 cycles of treatment, no significant differences were observed in endometrial thickness between groups, suggesting that all treatments are effective in balancing the effects of estradiol on endometrium. Regarding bleeding control, patients in Groups C and D showed a higher number of episodes of regular bleeding than patients in Groups A and B and fewer episodes of spotting. The better control of bleeding was associated with a higher treatment compliance in patients who received vaginal NP, with a larger percentage of women completing the study.

Conclusion Transdermal estrogen replacement therapy combined with 100 mg of micronized NP administered per vagina from the 14th day to the 25th day of each 28-day cycle leads to good cycle control and provides excellent patient satisfaction without serious side-effects. This therapy could be a treatment of first choice in early postmenopausal patients.

INTRODUCTION

Progestins are administered during estrogen replacement therapy in postmenopausal women, either continuously sequentially or combined, in order to prevent endometrial hyperplasia. Indeed, several studies have demonstrated that unopposed estrogen use is associated with a high risk (relative risk 2.1–5.7) of endometrial hyperplasia and adenocarcinoma and that the addition of a progestin for at least 10–14 days per month protects the endometrium from these modifications.

While the benefits of progestins in estrogen–progestrone replacement therapy (EPRT) are well recognized, data in the literature about the risks of their use are controversial and recent evidence has shown the importance of the choice of the progestin in terms of side-effect profiles. Natural progesterone (NP) does not bind to the androgen receptor and does not induce androgenic side-effects.

Micronized NP is chemically identical to progesterone of ovarian origin. The micronized formulation provides optimal progesterone bioavailability, which depends both on the size of the progesterone particles in suspension and the nature of the oily excipients. Indeed, orally administered micronized progesterone undergoes a significant hepatic first-pass effect, which results in a large amount of the absorbed steroid circulating as metabolites, primarily pregnanediol,
pregnenolone, pregnanediol, 20α-dihydroprogesterone and 17β-hydroxyprogesterone. The prospective, comparative Postmenopausal Estrogens/Progestin Intervention trial has recommended oral micronized progesterone as the first choice for opposing estrogen therapy in non-hysterectomized, postmenopausal women. Indeed, the micronized formulation administered orally once daily has been shown to be as effective as the synthetic progestins for controlling endometrial growth, but to have significantly fewer metabolic side-effects. Moreover, the use of NP in EPRT has recently been reported to be associated with a lower risk of breast cancer in comparison with synthetic progestins. Therefore, it seems that the use of NP may have some advantages over other progestins.

However, our group has recently performed a study on the oral administration of four different progestins (medroxyprogesterone acetate, 10 mg/day; nomegestrol acetate, 5 mg/day; dydrogesterone, 10 mg/day; micronized progesterone 200 mg/day) in association with transdermal estradiol in EPRT. We observed a lower incidence of regular withdrawal bleeding in patients receiving oral NP. Moreover, in this group, there was a higher incidence of episodes of irregular bleeding and spotting and the progesterone-associated bleeding took place significantly earlier in comparison to the other groups.

Given the hypothetical advantages of NP over other synthetic progestins, and considering the low compliance to EPRT in patients with irregular bleeding, it seemed interesting to evaluate which dose and which route of administration of NP leads to better cycle control.

The aim of the present study was to evaluate the effects on bleeding pattern of two different doses of NP administered per os or per vagina, in association with transdermal estradiol, in a continuous, sequential estrogen–progestin replacement therapy (CS-EPRT).

MATERIALS AND METHODS

Participants

From June 2008 to June 2009, 712 women, at least 12 months and no more than 36 months after spontaneous menopause, attending the Menopause Clinic of our Department, were assessed for eligibility to participate in the study. The inclusion criteria were: climacteric symptoms, body mass index < 30 kg/m²; absence of any significant pathology; absence of contraindications to EPRT; no previous use of hormonal drugs for climacteric-related symptoms.

The exclusion criteria were: neoplastic, metabolic and infectious diseases, a uterine size greater than 12 weeks’ gestation, any abnormality at bimanual pelvic examination, concomitant use of any hormonal drug, body mass index > 30 kg/m², cigarette smoking. An endometrial thickness > 4 mm or the presence of any endometrial abnormalities at transvaginal ultrasonography (TV-USG) were considered other exclusion criteria.

Upon admission, all women underwent a complete clinical evaluation, including physical (weight, height, blood pressure, heart beat), breast and gynecological examinations, TV-USG, mammography, Pap smear and biochemical and urinary determinations. In all subjects, the menopausal status was confirmed by levels of serum follicle stimulating hormone and estradiol in the postmenopausal range.

Informed consent and approval

Before entering into the study, the purpose of the protocol had been explained to all women and a written informed consent was obtained by all subjects enrolled. The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation and the study was approved by our Institutional Review Board.

Hormones used and doses

Among the 712 women evaluated, 524 did not meet inclusion criteria, 41 refused to participate and 47 were not included for other reasons, leaving 100 patients that were included in the study. Using a computer-generated randomization list, the 100 subjects were randomized into four groups, each of 25 women.

All women received transdermal 17β-estradiol (Dermestril 50, Rottapharm, Italy) at the dose of 50 µg/day, combined with two different doses of micronized NP (Prometrium, Rottapharm, Italy) administered at home per os or per vagina from the 14th day to the 25th day of each 28-day cycle. Groups A and B received NP per os at the dose of 100 mg/day and 200 mg/day, respectively. Groups C and D received NP per vagina at the dose of 100 mg/day and 200 mg/day, respectively.

These doses are those generally used in common practice. The duration of the treatment was 12 cycles, each of 28 days. Both women and researchers were not blinded to the treatment.

The primary outcome of the present study was to evaluate the effects on bleeding pattern of these two treatments in terms of regular progesterone-associated bleeding, episodes of amenorrhea, episodes of irregular bleeding, episodes of spotting, day of onset of regular progesterone-associated bleeding, and duration of regular progesterone-associated bleeding. The secondary outcome was endometrial thickness determined by means of TV-USG. At the beginning of the study and after 12 cycles of treatment, endometrial thickness was evaluated by TV-USG in all patients.

TV-USG scanning after 12 cycles of treatment was performed immediately after progestinic withdrawal bleeding. This, indeed, represents the best timing for
monitoring endometrial thickness during sequential HRT regimens. Ultrasonographic scans were performed by the same experienced operator (C.D.C.). Endometrial thickness was measured at the thickest part in the longitudinal plane, by scanning from cornua to cornua. The poorly echogenic layer surrounding the highly echogenic endometrium was not included in the measurement. Endometrial thickness was evaluated to determine the mean of three measurements and excluding the possible endometrial fluid from the measurement.

Data collection

Patients were asked to record, in a daily diary, the occurrence of vaginal bleeding or any adverse event. Patients were asked to record, in the same diary, the days of the application of each patch, the days of administration of NP and the exact moment of bleeding onset.

At the end of the study, the daily diaries were examined and the following parameters were calculated in order to evaluate the regularity of bleeding: incidence of regular progesterone-associated bleeding, incidence of amenorrhea, incidence of spotting and irregular bleeding, day of onset of progesterone-associated bleeding (calculating as day 1 the first day of progesterone administration), duration of each progesterone-associated bleeding. Regular progesterone-associated bleeding was defined as any bleeding occurring toward the end of or immediately after NP administration. Amenorrhea was defined as lack of bleeding after progesterone withdrawal lasting until the next progesterone administration; all other bleedings were considered as irregular. When irregular bleeding was so mild as not to require the use of sanitary pads, it was classified as spotting.

Statistical analysis

Statistical analysis was performed using the SPSS 13.0 (SPSS Inc.; Chicago, IL, USA).

On the basis of our preliminary experience and having as primary end-point the incidence of regular progesterone-associated bleeding, we calculated that, in order to detect a significant difference (p < 0.05) between groups, with a power of 95%, at least 190 cycles for each group should be evaluated. The enrolment of 25 patients for each group would therefore guarantee enough statistical power to the study.

A Shapiro–Wilk’s test was performed to evaluate the distribution of data for all parametric variables. Age and body mass index showed a normal distribution. Therefore, differences between groups at the beginning of the study for these variables were evaluated by ANOVA, followed by the Newman and Keuls post-hoc procedure. The data for endometrial thickness and time from menopause showed a non-normal distribution and therefore the differences among the four groups for this variable were calculated by the Mann–Whitney test. Differences between groups for incidence of regular cycles, amenorrhea, spotting and irregular bleeding were evaluated by the \( \chi^2 \) test. Differences between groups for the day of onset of bleeding and for the duration of bleeding were evaluated by the Wilcoxon rank sum test.

Statistical significance was set at p < 0.05. Data were expressed as mean ± standard deviation (SD) or median [range], as appropriate.

RESULTS

Eighty of the 100 enrolled subjects completed the study. Drop-outs were due to lack of compliance to the treatment for 14 subjects (six, five, one and two women in Groups A, B, C and D, respectively) and to missed control for personal reasons for six subjects (three women in Group C and three women in Group D). Data from these patients were not considered in the final calculation and are reported in Table 1. The treatment was well tolerated in all groups. No relevant drug-related, adverse events were observed during the study.

The characteristics of the subjects studied are reported in Table 2. At the beginning of the study, there were no significant differences among the groups in age, time since menopause and body mass index (Table 2).

Similarly, at baseline, no significant differences were detected between the four groups in median endometrial thickness (3.0 [2.5–3.3] mm in Group A, 3.1 [2.9–3.5] mm in Group B, 2.4 [1.8–3.7] mm in Group C and 3.0 [1.9–3.6] mm in Group D) (Table 2). After 12 cycles of treatment, no significant differences were observed in endometrial thickness between groups (4.1 [3.3–4.8] mm in Group A, 3.9 [2.8–4.9] mm in Group B, 3.9 [3.1–5.0] mm in Group C and 3.5 [2.0–3.9] mm in Group D). Endometrial thickness was <6 mm in all cases.

Data on bleeding characteristics are reported in Table 3. From a total of 960 treatment cycles, 15 cycles were not evaluable because of incomplete menstrual diaries (four, three, and five cycles in Groups A, B, C and D, respectively). Among the remaining 945 cycles,
we observed regular progesterone-related bleeding in 627 cycles (66.3%), 76 episodes of amenorrhea (8.0%), 103 episodes of irregular bleeding (10.9%) and 139 episodes of spotting (14.7%).

Regular progesterone-associated bleeding was significantly higher in Groups C and D than in Group A (194 (77.9%) and 163 (69.4%) vs. 125 (55.8%); \(p < 0.01\) and \(p < 0.01\), respectively) but significantly lower in Group D than in Group C (163 (69.4%) vs. 194 (77.9%); \(p < 0.05\)). Regular progesterone-associated bleeding was also significantly higher in Group C than in Group B (194 (77.9%) vs. 145 (61.2%); \(p < 0.01\)).

Episodes of amenorrhea were significantly higher in Group D than in all other groups (35 (14.9%) vs. 11 (4.9%), 18 (7.6%) and 12 (4.8%), respectively).

Episodes of irregular bleeding were significantly higher in Group A than in all other groups (43 (19.2%) vs. 28 (11.8%), 20 (8.0%) and 12 (5.1%), respectively; \(p < 0.05\), B vs. A; \(p < 0.01\), C vs. A; \(p < 0.01\), D vs. A). No significant differences were observed between Groups C and D. Episodes of irregular bleeding were also significantly lower in Group D than in Group B (\(p < 0.05\)).

Episodes of spotting were significantly lower in Groups C and D than in Groups A and B (23 (9.2%) and 25 (10.6%) vs. 45 (20.0%) and 46 (19.4%), respectively). No significant differences were observed between Groups C and D.

The day of onset of regular bleeding and the duration of regular progesterone-associated bleeding are reported in Table 3. No significant differences were observed for these parameters among the groups.

**DISCUSSION**

Data from several studies have demonstrated that administration of NP has fewer metabolic side-effects than administration of synthetic progestins. Moreover, it has been recently observed that the use of NP is safer for breast cancer risk in comparison to other progestins. However, for pharmacological and clinical reasons, the use of NP until now has had a limited place in clinical practice. Indeed, among the various drugs available with progestative action, NP is largely the weakest.

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### Table 2: Clinical characteristics of patients at the beginning and the end of the study. Data are reported as mean ± standard deviation, or median [range]

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.2 ± 1.9</td>
<td>51.1 ± 2.3</td>
<td>51.2 ± 2.0</td>
<td>51.9 ± 2.4</td>
</tr>
<tr>
<td>Months since menopause</td>
<td>14.5 ± 2.3</td>
<td>13.9 ± 2.1</td>
<td>14.2 ± 1.5</td>
<td>13.7 ± 1.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 0</td>
<td>24.5 ± 2.2</td>
<td>24.9 ± 2.3</td>
<td>25.2 ± 2.0</td>
<td>24.2 ± 2.5</td>
</tr>
<tr>
<td>After 12 cycles</td>
<td>24.6 ± 2.8</td>
<td>24.9 ± 2.5</td>
<td>25.7 ± 2.1</td>
<td>24.1 ± 2.9</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time 0</td>
<td>3.0 [2.5–3.3]</td>
<td>3.1 [2.9–3.5]</td>
<td>2.4 [1.8–3.7]</td>
<td>3.0 [1.9–3.6]</td>
</tr>
<tr>
<td>After 12 cycles</td>
<td>4.1 [3.3–4.8]*</td>
<td>3.9 [2.8–4.9]*</td>
<td>3.9 [3.1–5.0]*</td>
<td>3.5 [2.0–3.9]</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. time 0

### Table 3: Characteristics of the bleeding patterns. Data are given as n (%) or median [range]

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment cycles evaluated (n)</td>
<td>224</td>
<td>237</td>
<td>249</td>
<td>235</td>
<td>945</td>
</tr>
<tr>
<td>Treatment cycles not evaluated (n)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Cycles with regular progesterone-associated bleeding, n (%)</td>
<td>125 (55.8)</td>
<td>145 (61.2)</td>
<td>194 (77.9)</td>
<td>163 (69.4)</td>
<td>627 (66.3%)</td>
</tr>
<tr>
<td>Episodes of amenorrhea, n (%)</td>
<td>11 (4.9)</td>
<td>18 (7.6)</td>
<td>12 (4.8)</td>
<td>35 (14.9)</td>
<td>76 (8%)</td>
</tr>
<tr>
<td>Episides of irregular bleeding, n (%)</td>
<td>43 (19.2)</td>
<td>28 (11.8)</td>
<td>20 (8.0)</td>
<td>12 (5.1)</td>
<td>103 (10.9%)</td>
</tr>
<tr>
<td>Episodes of spotting, n (%)</td>
<td>45 (20.0)</td>
<td>46 (19.4)</td>
<td>23 (9.2)</td>
<td>25 (10.6)</td>
<td>139 (14.7%)</td>
</tr>
<tr>
<td>Day of onset of regular progesterone-associated bleeding</td>
<td>11 (8–14)</td>
<td>12 (9–14)</td>
<td>12 (8–15)</td>
<td>13 (9–16)</td>
<td></td>
</tr>
</tbody>
</table>
According to these considerations, data from a previous study by our group seem to suggest a lower secretory effect of NP in comparison to the other progestins (medroxyprogesterone acetate, nomegestrol and dydrogesterone), leading to a greater number of irregular bleeding episodes. In the present study, we investigated the bleeding pattern in four groups of women receiving transdermal estrogen replacement therapy combined with two different doses of micronized NP administered per os or per vagina from the 14th day to the 25th day of each 28-day cycle. No significant differences were observed in endometrial thickness between the groups, suggesting that all treatments are effective in balancing the effects of estradiol on endometrium.

Regarding bleeding control, data from this study seem to suggest that the vaginal administration of NP was better than oral administration, with higher episodes of regular bleeding and fewer episodes of spotting. The better control of bleeding was associated with a higher treatment compliance in patients who received NP transvaginally, with a larger percentage of women completing the study (respectively, 96% and 92% in Groups C and D vs. 76% and 80% in Groups A and B).

Better compliance in patients treated with vaginal progesterone may be explained also by the consideration that oral administration of progesterone, because of the production of high levels of metabolites during the first pass in the liver, may cause side-effects on the central nervous system (most notably drowsiness, dizziness, sleep disturbances, etc.) known to interfere with long-term patient compliance.

The good bleeding control after vaginal administration confirms the hypothesis that the vaginal route of administration allows a preferential distribution of progesterone to the uterus. Indeed, Cicinelli and colleagues have demonstrated that, after vaginal administration, progesterone levels are significantly higher in the uterine arterial blood than in the radial artery. This selective distribution is probably mediated by a countercurrent transfer of progesterone in the paracolpium and parametrium. In Group D, we observed a higher rate of amenorrhea, probably due to the same mechanism.

In conclusion, data from our study seem to suggest that transdermal estrogen replacement therapy combined with 100 mg of micronized NP administered per vagina from the 14th day to the 25th day of each 28-day cycle leads to good cycle control and provides excellent patient satisfaction in the absence of important side-effects. This therapy could be a treatment of first choice in early postmenopausal patients, considering also the possible side-effects of synthetic progestin, notably on the breast and on the cardiovascular system. The starting treatment dose of progesterone should be the lower (100 mg/day), to avoid the risk of amenorrhea. Additional prospective studies are required to verify these preliminary findings over longer periods.

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References