A prospective randomized controlled study comparing two doses of gestodene in cyclic combined HRT preparations on endometrial physiology

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Postmenopausal women taking oestradiol 17-β 2 mg daily were randomized to receive either 25 or 50 µg gestodene from day 17 to 28 of the cycle in a double-blind study. Placental protein P14 (PP14) and CA 125 concentrations in uterine flushing, endometrial morphology and irregular bleeding after 12 cycles of study were observed. Eleven and 12 women in the 25 and 50 µg groups respectively completed the study. There were no significant differences in pre-treatment biochemical and morphological indices between the groups. The median PP14 concentration increased from 332 to 5800 ng/ml (P < 0.001) and from 145 to 27 160 ng/ml (P < 0.001) in the 25 and 50 µg gestodene groups respectively. No between-group significant rise of PP14 was observed. Similarly, no significant change was seen between the initial and post-treatment concentrations of CA 125 for either group. All biopsies were atrophic at inception of the study, and both regimens produced secretory endometrial transformation in the majority of biopsies. No between-group difference was observed in the morphometric indices measured, or any significant correlation between the concentrations of PP14 or CA 125 and morphology. The mean number of days of withdrawal bleeding (3.8 and 4.2 days for 25 and 50 µg respectively) were similar. In conclusion, both regimens produced a significant rise in uterine flushing concentrations of PP14, but not CA 125. PP14 is a sensitive biochemical marker in the assessment of endometrial response to hormone replacement therapy.

Key words: CA 125/cyclical sequential oestrogen–gestodene therapy/endometrial morphology/placental protein 14/postmenopausal women

Introduction

Although monthly withdrawal bleeding and irregular bleeding induced by conventional hormone replacement therapy (HRT) are important reasons why patients discontinue treatment (Nachtigall, 1990; Okon et al., 1996), current understanding of the mechanisms underlying bleeding with HRT is limited. The usual pattern of bleeding at the end of or after the progestogen phase of treatment consists of 2–4 days, although a median of 7 days of bleeding has been reported (Al-Azzawi et al., 1994). Variations from this pattern may indicate pathology, inadequate oestrogen or progestogen dose, or poor compliance. Understanding of the mechanisms of bleeding during HRT use is important, as this could lead to the introduction of products with reduced risks of unpredictable bleeding which would probably induce a better acceptance of HRT. In normal menstruation, two theories—vasoconstrictor and inflammatory—have been advanced to explain the mechanism (Salamonsen et al., 1999).

Oestrogen plays the central role of priming the endometrium, and allows progesterone to exert its effects (Li et al., 1992a). In the presence of atrophic endometrium due to insufficient oestrogen priming, the addition of a progestogen may have no effect or render the endometrium unstable and lead to abnormal bleeding. Bleeding may also depend on the type of progestogen used, as this could influence the endometrial vascular changes (Casanas-Roux et al., 1996). Levonorgestrel, in particular, may have an additional effect on endometrial vasculature to produce well-developed arterioles and dilated sinusoids, which may account for the higher incidence of breakthrough bleeding with this progestogen (Graham and Fraser, 1982).

Currently, the most commonly used progestogen in HRT preparations is the 19-nortestosterone, norethisterone, and this has significant androgenic activity. In recent years newer progestogens with less androgenic effect and with better cycle control have been developed and used for oral contraception. Such new progestogens include desogestrel, gestodene and norgestimate (Speroff and DeCherney, 1993).

Gestodene is a 19-nortestosterone progestogen that has a number of theoretical advantages. One of its most important properties is efficient cycle control. In view of its potency,
lower doses will reduce androgenic activity whilst producing secretory endometrium, particularly in women with hirsutism and acne. Previous pilot safety evaluations of gestodene in postmenopausal HRT have suggested that 25 and 50 µg of gestodene in cyclical regimens for 12 days both caused a secretory endometrial transformation, without untoward changes such as endometrial hyperplasia during the period of follow-up (Schering Reports 91056, 9291 and 8946). Previous systematic clinical studies of dose-related, progesterone-related effects at constant oestrogen doses in artificial cycles showed significant differences in the mitotic activity, volume fraction of gland occupied by gland cells and volume fraction of endometrium occupied by glands, together with a significant reduction of the number of supra- and subnuclear secretory vacuoles (Li et al., 1992b).

The study of endometrial function has been by histological examination of endometrial biopsy specimens using either simple light microscopy or more refined histological techniques such as morphometry (Li et al., 1988), histochemistry (Klentzeris et al., 1991), immunohistochemistry (Bell et al., 1985) and computer-assisted three-dimensional evaluation (Casanas-Roux et al., 1996). These techniques have permitted a more detailed study of the various components of the endometrium. A recent development has been the study of endometrial physiology by analysis of uterine protein content (Li et al., 1993a) and patterns of distribution of these proteins (Beier-Hellwig et al., 1989) in endometrial secretions obtained by direct aspiration or by the technique of uterine flushing (Li et al., 1993b).

One of the endometrial proteins which reflects the secretory activity of the endometrium in premenopausal women is placental protein 14 (PP14). Concentrations of this glycoprotein, which is secreted by the endometrium, start to rise in the luteal phase and reach their highest concentrations in the plasma and uterine fluids in the luteal phase (Joshi et al., 1986; Julkunen et al., 1986; Li et al., 1993a). Another glycoprotein produced by the endometrium is CA 125 (Jacobs and Bast, 1989), concentrations of which in uterine flushings have been shown to correlate with uterine PP14 (Dalton et al., 1995). It has been shown previously that plasma PP14 concentrations rise in women receiving HRT (Li et al., 1992c), but to date few data are available regarding the concentrations of these two proteins in uterine flushings from women receiving HRT.

The objective of this study was to compare two different doses of gestodene in a sequential oestrogen/progestogen combination using morphological and biochemical methods. In addition, the morphometric and biochemical changes were compared to determine how well the changes correlated to each other. This study formed part of a double-blind, multicentre trial evaluating the safety of 25 and 50 µg gestodene in combination with 2 mg oestradiol-17β as a novel HRT; however, the data on PP14 and CA 125 were collected only at this centre.

Materials and methods

Subjects
Thirty-three postmenopausal women, defined as those women under 65 years of age who had a minimum of 12 and a maximum of 72 months of amenorrhoea, with a serum FSH concentration >20 IU/l and plasma oestradiol concentration ≤30 pmol/l, intact uterus and requesting HRT, were entered into this study. The women were recruited from among patients referred by general practitioners to the Jessop Hospital for Women for HRT advice. The volunteers underwent routine blood investigations for liver and renal function and for haematological indices. Those women with abnormal results were excluded. The volunteers were randomized into two groups by computer-generated codes. Allocation of the randomization number was linked in a chronological ascending manner to the sequence of arrival of the volunteers. Drop-outs were not replaced. A sealed emergency envelope containing treatment details for the investigators was issued for each subject in the study. The seal was only broken in the case of medical emergency and none of the women that completed the study had their envelopes opened. Both groups were given oestradiol 17β 2 mg daily. Gestodene was added from day 17 to 28 (12 days) of the 28-day treatment cycle, with one group receiving 25 µg daily and the other 50 µg daily. None of the women had taken any steroid hormones for 3 months before entering the study. The study was approved by the local ethics committee, and each of the volunteers provided their informed consent before entry.

Uterine flushing
This was performed as an out-patient procedure on day 25 or 26 of the treatment cycle using the Pipelle endometrial sampler (Laboratoire CCD, Paris, France) (Figure 1). None of the women had started bleeding before this procedure was performed. The method previously described (Okon et al., 1998) was used. With the volunteer in the dorsal position, the thighs and legs in flexion, pelvic examination was performed to exclude any gross pelvic pathology and to determine the position and size of the uterus. Following asepsis of the external genitalia using Savlon® (0.015% w/v chlorhexidine gluconate, 0.15% w/v cetrimide; Zeneca, Wimslow, Cheshire, UK), a bivalve speculum was inserted into the vagina, exposing the cervix, which was then cleaned with Savlon. A Pipelle endometrial sampler that had been preloaded with 1 ml of sterile physiological saline (0.9% NaCl) was slowly introduced into the uterus. After about 30 s, the tip of the sampler was withdrawn by about 2 cm into the lower part of the uterine cavity, after which the saline solution was aspirated slowly through the vacuum mechanism of the Pipelle sampler. The plunger was withdrawn in increments of ~2 cm over a period of ~30 s. A volume of between 0.8 and 1 ml was consistently aspirated. The aspirate was immediately centrifuged at 2200 g for 5 min and the supernatant stored at ~20°C for assay of PP14 and CA 125.
**Endometrial biopsy and morphology**

These were performed at inception and on day 25 or 26 of the 12th treatment cycle, and none of the women had started withdrawal bleeding at the time of the biopsy. The endometrial biopsy specimen was obtained by use of the same Pipelle sampler used for flushing. This was reintroduced gently until the tip rested on the uterine fundus, signified by an increase in resistance after that of the internal os of the cervix. The plunger was then withdrawn and strips of endometrial tissue were taken by gentle sliding movements. Each biopsy was fixed immediately in glutaraldehyde in cacodylate buffer (pH 7.4) and sent to the pathology laboratory at the Royal Hallamshire Hospital for processing and analysis. The fixed tissue was embedded in paraffin wax and stained with haematoxylin and eosin before being sectioned for examination by light microscopy. The endometrial biopsies were examined histologically using published dating criteria (Noyes et al., 1950) and morphometric analysis (Li et al., 1988).

The morphometric features measured were: (i) number of mitoses per 1000 gland cells; (ii) amount of secretion in gland lumen (score 0 to 3); (iii) volume fraction of gland occupied by gland cell; (iv) volume fraction of endometrium occupied by gland; (v) volume fraction of gland cell occupied by nucleus; (vi) number of supranuclear secretory vacuoles per 100 gland cells; (vii) number of subnuclear secretory vacuoles per 100 gland cells; and (viii) number of mitoses per 1000 stromal cells. For each measurement a minimum of 20 fields were examined from each slide.

**PP14 assay**

PP14 was measured by radioimmunoassay using the method described previously (Bolton et al., 1987). PP14 was iodinated by the chloramine-T method, and the resulting tracer was purified using a column of ConA Sepharose. For the assay, 100 µl of 1 ng/ml PP14 tracer and 100 µl of standards or sample were incubated at room temperature for 24 h with 100 µl antiserum, at a dilution to bind 45% of the added tracer. The antibody-bound tracer was separated from the unbound using Amerlex-MMT magnetic separating reagent (Amersham International, Little Chalfont, Bucks, UK). The lower sensitivity of the assay was 3 ng/ml, and the intra- and inter-assay coefficients of variation were below 10% at a concentration of 3 ng/ml.

**CA 125 assay**

CA 125 concentrations in serum and uterine flushing were measured at the Immunology Department of the Northern General Hospital, Sheffield using an enzyme-linked immunosorbent assay (ELISA)-CA 125 11 kit (CIS Bio International, Cedex, France), which is a two-site immunoradiometric assay. The CA 125 was adsorbed onto the solid phase by antibody ELISA-CA 125 11 and then quantified using radioiodinated OC125 antibody as tracer. All assays were carried out in duplicate and performed according to the manufacturer’s instructions. The lower and upper limits of detection were 0.5 and 500 IU/ml respectively, and the inter- and intra-assay coefficients of variation were <5% at these concentrations.

**Study follow-up and menstrual calendar**

The volunteers were requested to record tablet taking and bleeding (if any) on a daily basis on a diary card provided. The women were reviewed every 3 months, and at the end of each 12 months the uterine flushing and the endometrial biopsy were repeated and analysis performed as described previously.

**Analysis of data**

In view of the variability in concentrations of PP14 and CA 125, the data were log transformed before analysis. Concentrations of PP14 and CA 125 and the morphometric indices in the groups were compared using the Mann–Whitney (non-parametric) U-test.

### Results

Thirty-three women started the study, but 10 withdrew before completion of the first year of treatment and were therefore excluded from the analysis because they would neither have had a uterine flushing nor biopsy after 12 cycles of treatment. The main reasons given for withdrawal were hormone-related side effects such as retention of fluid, weight gain, depression and breast tenderness. None of the women withdrew for bleeding problems, although this occurred in the other centres based in the UK, Germany, Belgium, the Republic of Ireland and The Netherlands. Of the 23 women left in the study in the first year, 11 were in the 25 µg group and 12 in the 50 µg gestodene treatment group. Two groups were identified for analysis on the basis of the dose of gestodene in the preparation. The age distribution and baseline interval from menopause to entry into study were similar between the treatment groups (Table I). There was no significant difference in the groups for age, body mass index or interval from the menopause.

### Table I. Baseline demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gestodene dose</th>
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<tr>
<td></td>
<td>25 µg (n = 12)</td>
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<tr>
<td>Age (years)§</td>
<td>51.6 ± 4.0</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>45–58</td>
</tr>
<tr>
<td>Interval since menopause (months)§</td>
<td>35.8 ± 20.0</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)§</td>
<td>26.5 ± 4.1</td>
</tr>
</tbody>
</table>

§Values are mean ± SD. BMI = body mass index.

Biochemistry

Figure 2 (box-plot) shows the concentrations of PP14 in uterine flushing at inception and after 12 months of study HRT. In the 25 µg gestodene group there was a significant increase in the median concentration of PP14 from a pre-treatment value of 332 ng/ml to 5800 ng/ml after treatment (P < 0.001). Similarly, in the 50 µg gestodene group, there was a significant rise in median concentration from 145 ng/ml pre-treatment to 332 ng/ml after treatment (P < 0.001). The median uterine flushing PP14 concentration was increased 30-fold, from 304 ng/ml before study to 8950 ng/ml after treatment. However, there was no significant difference in the rise in PP14 concentration between subjects in the 25 and 50 µg gestodene groups.

The pre-treatment median serum concentration of PP14 in the 25 µg gestodene group was 11.7 ng/ml, and this rose to 20.1 ng/ml after 1 year of treatment (P < 0.05). However, in the 50 µg gestodene group, the increase in median serum PP14 concentration was not significant (from 14.0 to 15.6 ng/ml). A comparison of the treated groups showed no significant difference in serum PP14 concentrations between those subjects receiving 25 or 50 µg gestodene.

The median serum concentrations of CA 125 pre and post...
Effect of oestrogen/gestodene on endometrial physiology

Figure 2. Uterine flushing concentrations of PP14 in postmenopausal women before and after treatment with gestodene/oestradiol. The box indicates the lower and upper quartiles, and the central line the median. The points at the end of the bars represent the 2.5% and 97.5% values. **Significantly different to pre-treatment ($P < 0.01$). pre25, post25, pre50 and post50 = pre- and after 12 months of treatment with 25 µg gestodene ($n = 11$) and 50 µg ($n = 12$) gestodene.

12 cycles of treatment in the 25 µg gestodene group were 16.0 and 15.5 IU/l respectively, while the corresponding values for the 50 µg gestodene group were 15.5 and 14.0 IU/l. There was no significant change in post-treatment plasma concentrations of CA 125 within either group, or between the treatment groups. The median uterine flushing concentrations of CA 125 pre and post 12 cycles of treatment in the 25 µg gestodene group were 45 600 and 41 360 IU/l respectively. No significant rise was observed between the pre- and post-treatment uterine flushing concentrations of CA 125 in either the 25 or 50 µg gestodene groups, or between treatment groups.

Bleeding patterns
Vaginal bleeding was defined as any appearance of blood in the vagina (regardless of amount), or any requirement for sanitary towels. Bleeding during treatment with HRT was classified as light (less than bleeding associated with normal menstruation), moderate (like normal menstruation) and heavy (more than normal menstruation). Bleeding was regular if it occurred between day 23 of the current cycle and day 4 of the subsequent cycle, and irregular for all other episodes of vaginal bleeding with or without regular bleeding. Bleeding data were not complete for two women in the 50 µg group; hence these were excluded from the analysis. The nature of bleeding before the menopause was based on patient recall. On average, bleeding was light to normal in the two groups of HRT regimen compared with the nature of bleeding before the menopause. There was no difference in the pattern of bleeding between the two preparations.

Figure 3 shows the pattern of menstruation in women taking 25 and 50 µg gestodene HRT preparations. The mean number of days of withdrawal bleeding in women taking 25 µg gestodene was 3.8 days, while that for women taking 50 µg was 4.2 days. There was no statistically significant difference between the two groups. Most of the bleeding occurred between day 28 and day 4 of the subsequent cycle. One subject who was taking the 25 µg preparation experienced an episode of irregular bleeding that occurred within the first 6 months of the trial. The overall multicentre regular or no bleeding was 80% for 25 µg gestodene and 74% for 50 µg, whilst the rest were irregular for each group.

Endometrial morphology
The response of the endometrium was assessed using published criteria (Noyes et al., 1950) (Table II) and seven morphometric indices (Johannisson et al., 1982; Li et al., 1987). Atrophy was observed in all subjects at the inception of the study. Insufficient tissue was obtained for analysis in three women at the end of the 12th cycle (in one woman in the 25 µg group, and in two women in the 50 µg gestodene groups). There was secretory transformation of the endometrium in the majority of biopsies from both treatment groups.

The mean (± SD) glandular secretory activity was 1.0 ± 0.4 for the 25 µg gestodene and 0.9 ± 0.5 for the 50 µg. There was no statistically significant difference in gland secretory activity between the two groups. Similarly, there was no difference in other morphometric indices in the two groups (Table III). Comparison of the morphometric indices on treatment showed no significant difference in endometrial response between those on 25 µg gestodene and those on 50 µg, using
Oestrogen monotherapy has been shown to be associated with menopausal endometrial secretory response, which may vary among individuals. The risk of endometrial cancer (Sturdee, 1994) may have affected the PP14 concentrations. This difference in bioavailability at the endometrial level, though not reflected in the histological biopsies, may account for the observed variation in uterine washing concentrations of PP14. To what extent these variations in endometrial level bioavailability and receptor-mediated responses affect endometrial PP14 production and secretion are speculative. Finally, it is possible that the postmenopausal endometrial secretory response may vary among women. It could be argued that the sampling technique could introduce this variation, but there was no change either in technique or in the person taking the samples (M.A.O.).

<table>
<thead>
<tr>
<th>Morphometric index</th>
<th>Gestodene group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>25 µg (n = 10)</td>
<td>50 µg (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glandular secretory activity</td>
<td>1.0 (1.0 ± 0.4)</td>
<td>1.0 (0.9 ± 0.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Supranuclear vacuoles/100 gland cells</td>
<td>0.0 (1.2 ± 2.6)</td>
<td>0.0 (1.1 ± 1.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Subnuclear vacuoles/100 gland cells</td>
<td>0.0 (1.5 ± 1.6)</td>
<td>0.0 (0.7 ± 1.4)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mitosis/1000 glandular cells</td>
<td>0.0 (0.0 ± 0.0)</td>
<td>0.0 (0.1 ± 0.5)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mitosis/1000 stromal cells</td>
<td>0.0</td>
<td>0.0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>vv:G/E (%)</td>
<td>18.1 (17.3 ± 3.9)</td>
<td>16.6 (17.4 ± 4.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>vv:G/C (%)</td>
<td>68.7 (69.1 ± 3.8)</td>
<td>68.1 (69.0 ± 5.9)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are median (mean ± SD).
vv:G/E = ratio of gland to endometrium; vv:G/C = ratio of gland cell to gland.
NS = no statistically significant difference.

the specified morphometric indices. Moreover, there were no features of endometrial hyperplasia in any of the samples over the study period. Neither were there any features of endometrial hyperplasia in any of the samples studied, which suggests that both regimens are protective over the 12-cycle period studied. No statistical correlation was observed between the biochemical markers and the morphological indices.

Discussion

Oestrogen monotherapy has been shown to be associated with an increased incidence of endometrial hyperplasia, and also an increased risk of endometrial carcinoma. The addition of progestogen to oestrogen therapy has succeeded in reducing the risks of endometrial cancer (Sturdee et al., 1994; Lobo, 1995), which can remain elevated for a decade after cessation of HRT (Green et al., 1996). In the current study the biochemical markers PP14 and CA 125 and endometrial morphometry were used to compare the response of the endometrium to two regimens of oestradiol/gestodene combination in postmenopausal women. Although these two proteins have been studied extensively, their use as markers of endometrial status in postmenopausal women has not been fully elucidated. Previously, it has been observed that PP14 concentrations in the endometrial fluid are reduced in postmenopausal women, and that endometrial CA 125 appears to be independent of menstrual status (unpublished data). It has also been reported that measurement of PP14 concentrations in uterine flushings may enable the different effects of various forms of HRT on endometrial function to be determined (Okon et al., 1998).

Biochemistry

In the current study, the uterine flushing concentrations of PP14 appear to show significantly greater variability than were observed in previous studies using period-free preparations (Okon et al., 1998). The reason for this variability may be measurement error, but this is unlikely as the PP14 assay was performed in paired samples. A more likely reason is the considerable inter-subject variations in pharmacokinetics for synthetic progestational compounds such as gestodene. Third, there may be a difference in the pharmacokinetics of the concurrently administered oestradiol 17β. Additionally, individual variations in the spectrum of oestrogenic, androgenic, antioestrogenic, glucocorticoid and the antimineralocorticoid effects of gestodene (Edgren, 1986) may have affected the PP14 concentrations. This difference in bioavailability at the endometrial level, though not reflected in the histological biopsies, may account for the observed variation in uterine washing concentrations of PP14. To what extent these variations in endometrial level bioavailability and receptor-mediated responses affect endometrial PP14 production and secretion are speculative. Finally, it is possible that the postmenopausal endometrial secretory response may vary among women. It could be argued that the sampling technique could introduce this variation, but there was no change either in technique or in the person taking the samples (M.A.O.).

There was no significant change in the concentration of CA 125 in uterine flushing during treatment. This supports earlier findings that there was no significant variation in the uterine flushing concentrations of this protein either during the menstrual cycle or during treatment with two different forms of period-free HRT (Okon et al., 1998).

Morphology

On the basis of the current results, no significant difference was observed between the various morphological measurements in the 25 and 50 µg gestodene groups, with both regimens producing secretory transformation in the majority of biopsies.

First, the differences in the endometrium constitute a spectrum, the elements blend very closely and may be difficult to discern. However, the chances of error were reduced by the use of the theoretically objective method of morphometric analysis. Nonetheless, to demonstrate a significant difference, the use of this method in a small number of patients requires it to be further improved. The only limitation of the observation is the fact that a relatively small number of biopsies were studied (10 in each group). However, it is still possible that there is indeed a difference between the two groups, which could be detected by a larger sample size; however, the
difference—even if it is present—is likely to be small. It was not possible to show any significant correlation between endometrial morphology and uterine flushings concentrations of PP14, most likely due to the relatively small number of subjects in the study or to the variability of PP14. Thus, larger subject numbers may be required to determine this relationship on a statistical basis.

Hence it appears that gestodene, in doses of 25 and 50 μg, produced a desirable endometrial response in the majority of subjects. The finding that 25 μg gestodene produced results similar to those achieved with 50 μg, means that the former dose level could be used to significant clinical advantages as it would produce fewer androgenic side effects.

**Bleeding**

Both preparations conferred good cycle control with scheduled withdrawal bleed. The degree and duration of loss did not differ from premenopausal levels; however, both the current subjects and those from other centres in this study menstruated for fewer days compared with the previously reported pattern of a mean of seven days (Habiba et al., 1996). Fewer women in our centre had irregular bleeding, and this difference may be the result of the smaller sample size compared with the overall result rather than the difference in bleeding patterns of the women at other centres. Irregular bleeding is one of the most important reasons given for withdrawal from HRT (Okon et al., 1996). This study shows desirable cycle control in the majority of subjects. Gestodene in preparations used for oral contraception is known to produce some of the best cycle control; however, its association in some studies with higher bleeding also needs to be considered.

In conclusion, both preparations of gestodene/estradiol 17β regimens produced a significant rise in uterine flushing concentrations of PP14, but not of CA 125. There was no significant difference in endometrial morphometry and biochemistry (PP14 and CA 125) between the two doses (25 and 50 μg) of gestodene used in the samples examined in the current study. As in previous studies, it seems that PP14 is a sensitive biochemical marker in the assessment of endometrial response to HRT in postmenopausal women.

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