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ORIGINAL ARTICLE

Endometrial morphology during hormone replacement therapy with estradiol gel combined to levonorgestrel-releasing intrauterine device or natural progesterone

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Objectives. To evaluate endometrial responses to three different forms of amenorrhea-inducing HRT in postmenopausal women.
Material and methods. Fifty-one postmenopausal women completing a one-year HRT trial with percutaneous estradiol gel containing 1.5 mg estradiol daily combined with a levonorgestrel-releasing intrauterine device (LNG-IUD) (n=18), or natural progesterone 100 mg daily orally (n=19) or vaginally (n=15) during 1-25 calendar days of each month. Endometrial thickness and uterine size were measured by transvaginal ultrasound, and endometrial cytology/histology was assessed from specimens taken by needle aspiration before the study and at 12 months.
Results. Before medication, the median endometrial thickness was 2.0 mm in the LNG-IUD group, 2.4 mm in the oral P group and 2.5 mm in the vaginal P group. At 12 months of therapy the respective values, 3.0, 2.7 and 2.4 mm, did not differ significantly from the initial values. LNG-IUD induced epithelial atrophy in all women, which was accompanied by stromal decidualization in 12 women. On the contrary, only four women in the oral P group and five women in the vaginal P group had an inactive or atrophic endometrium. The remaining cases were dominated by proliferative features. No hyperplasia was seen in any of the groups.
Conclusion: LNG-IUD appeared to be an effective method of counteracting the stimulatory effect of estrogen on the endometrium, whereas natural progesterone given orally or vaginally was not sufficiently effective in this function at the doses used. The vaginal and oral administrations of progesterone did not differ from each other in this respect.

Key words: amenorrhea-inducing HRT; endometrial cytology; endometrium; levonorgestrel-releasing IUD; menopause; uterus

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Menopausal hormone replacement therapy (HRT) with different treatment regimens has become widely used during the last decade. Pure estrogen therapy without progestin increases the risk of endometrial hyperplasia and cancer (1). Addition of progestin with an adequate dosage and duration to estrogen therapy protects the endometrium from these severe side-effects (2, 3). While sequential HRT has been extensively investigated, the data available on the long-term endometrial effects

Abbreviations:
HRT: hormone replacement therapy; LNG-IUD: levonorgestrel-releasing intrauterine device; FSH: follicle stimulating hormone; IGFBP-I: insulin-like growth factor binding protein-1.

of combined HRT aiming to induce endometrial atrophy are still limited (4).

As the endometrium is the only goal for progestin in HRT, intrauterine application of progestin seems logical. Indeed, an intrauterine levonorgestrel-releasing device (LNG-IUD) has been proved promising for this purpose (5–8). Another interesting alternative for an amenorrhea-inducing form of HRT is natural progesterone, which has no significant metabolic side-effects. According to the previous studies, progesterone administered orally during 25 calendar days gives a better bleeding profile than uninterrupted administration (9, 10). Vaginal administration of natural progesterone has not yet been studied in postmenopausal women, although it has been successfully used in fertile-aged women (11). This study aimed to evaluate the endometrial responses to LNG-IUD and natural progesterone administered orally or vaginally in postmenopausal women using percutaneous estradiol gel on a daily basis.

Material and methods

Sixty menopausal women contacted through an advertisement in the local newspaper were recruited as volunteers into this prospective one-year trial with three different forms of HRT in the Kainuu Central Hospital, Finland (8). Fifty-one of them completed the study, and their endometrial response during HRT could be studied. The eligible subjects had an intact uterus, no contraindications to HRT and a serum follicle stimulating hormone (FSH) concentration of more than 20 IU/l, and the time from their last natural menstruation was at least six months. Previous users of HRT underwent a wash-out period of at least two months and also had FSH >20 IU/l. The study design was approved by the ethical committee of the hospital.

The women were allocated alternatively into three groups in the order in which they entered the study, with the exception that an IUD was not inserted into nulliparous women (n=5). If a nulliparous woman was allocated into group I (IUD), she was placed in the next of the remaining two groups. Eligibility assessment was performed before allocation. All women accepted the treatment allocated to them.

The estrogen treatment was transdermal 17β-estradiol-containing gel (Estrogel®, Besins-Iscovesco, Paris, France), which contained 1.5 mg of estradiol per 2.5 g gel in all the three groups. This dose releases 150 μg estradiol into the circulation daily.

The regimens differed from one another in their type and route of progestin administration: 18 women in the first group had a LNG-IUD (Levonova®, Leiras Oy, Turku, Finland) inserted at the beginning of the study. The LNG-IUD releases 20 μg of levonorgestrel per 24 h for 5 years.

Nineteen women in the second group (oral P) used natural micronized progesterone capsules 100 mg with nutoil as a vehicle (Lugesteron®, Leiras, Turku, Finland) orally, and 15 women in the third group (vaginal P) took it vaginally during the 1st-25th calendar days of each month. In the latter group, the daily dose was raised to 200 mg in 12 women due to irregular bleeding.

The results concerning clinical compliance and bleeding profiles (8), and the endometrial expression of progestin-specific insulin-like growth factor binding protein-1 (12) have been published previously.

Endometrial samples were obtained by aspiration using the Pistolet method (13) before the treatment and at 12 months of trial. The cytological samples were prepared by the Sytotech centrifuge method and Papanicolaou stain. Samples containing enough tissue material were embedded into paraffin as histological preparations and stained by Hematoxylin-eosin. On the basis of a cytological examination only (n=49) or a combined cytological and histopathological examination (n=29), the endometrial morphology was classified into six groups:

1. endometrial atrophy and absence of mitoses in epithelial cells along with stromal decidualization,
2. inactive endometrium (atrophic or pseudostratified epithelium with no mitoses or stromal reaction),
3. partly proliferative (mostly inactive epithelium showing pseudostratified epithelium with mitoses in a small part),
4. mostly proliferative endometrium with mitoses in the epithelial cells (regarded as an estrogen effect),
5. secretory endometrium (if secretory changes in the endometrial glands could be seen), and
6. undiagnostic, if there was too little material for an evaluation.

Endometrial thickness (double-layer) and uterine dimensions (longitudinal × vertical length, square in cm) were measured using a Hitachi EUB 450 ultrasound with a transvaginal 6.5 Hz probe.

Results

Endometrial thickness measured by transvaginal ultrasonography before the treatment was similar in all groups (Table I). It was thicker than 5 mm in five women. The median endometrial thickness did not change considerably during the treatment in any group (Table I). At 3 months the endo-

metrium was thicker than 5 mm in six women: three in the LNG-IUG group and two in the oral P and one in the vaginal P group. At six months, there were two women in each group with an endometrium thicker than 5 mm. At twelve months, the figures were two, one and three in the three groups, respectively. Uterine size did not grow remarkably in any of the women during the trial (Table I).

The endometrial sample taken before the treatment was diagnostic in all but one woman. The morphology of the endometrium was atrophic or inactive in 46 women and four women had a partially proliferative endometrium. At 12 months' treatment, all but two samples (n=49) contained an adequate amount of cells for cytological analysis and 29 samples contained enough material for a histological evaluation, too (Table II).

Epithelial atrophy accompanied by stromal decidualization was the dominating finding in the LNG-IUD group (Table II). The endometrium was atrophic in five women. In one woman an endometrial polyp removed by dilatation and curettage was the reason for an endometrial thickness of 13 mm. In this case, histological examination revealed atrophic epithelium with stromal decidualization and in the polyp also small fragments of proliferative endometrium, but no hyperplasia. There might have been a polyp already before the treatment as the pre-treatment endometrial thickness of this woman was 5 mm.

Table I. Endometrial thickness (double-layer, mm) and uterine dimensions (longitudinal × vertical length, square in cm) during the one-year follow-up. The figures are medians (min, max)

	GROUP		
	LNG-IUD n=18	Oral P n=19	Vaginal P n=14
Endometrial thickness			
0 months	2.0 (0.6, 5.0)	2.4 (1.3, 7.9)	2.5 (0.9, 4.0)
3 months	3.6 (2.3, 8.2)	2.9 (1.7, 14.0)	2.5 (1.0, 5.1)
6 months	3.2 (2.6, 7.1)	2.7 (1.0, 6.8)	3.0 (0.9, 6.5)
12 months	3.0 (1.2, 13.2)	2.7 (1.5, 6.6)	2.4 (1.3, 7.0)
Uterine dimension			
0 months	18.8 (11.2, 27.4)	14.1 (4.2, 24.2)	15.6 (8.3, 25.8)
3 months	20.9 (12.6, 36.9)	17.9 (8.9, 22.8)	19.8 (9.5, 37.7)
6 months	22.4 (16.3, 29.5)	17.3 (11.0, 23.9)	19.0 (13.2, 52.3)
12 months	19.1 (14.0, 29.6)	21.7 (9.6, 26.8)	16.6 (12.0, 49.1)

Table II. Endometrial morphology at 12 months

	GROUP		
	LNG-IUD n=18	Oral P n=19	Vaginal P n=14
Epithelial atrophy and stromal decidualization	12 (2)*	–	–
Inactive endometrium	5	4	5
Partly proliferative	–	5	1
Mostly proliferative	–	8 (2)	7 (1)
Secretory	–	1	1 (1)
Undiagnostic sample	1	1	–

* One patient also had a partly proliferative endometrial polyp, see text. The number of women with endometrial thickness of more than 5mm is given in parentheses.

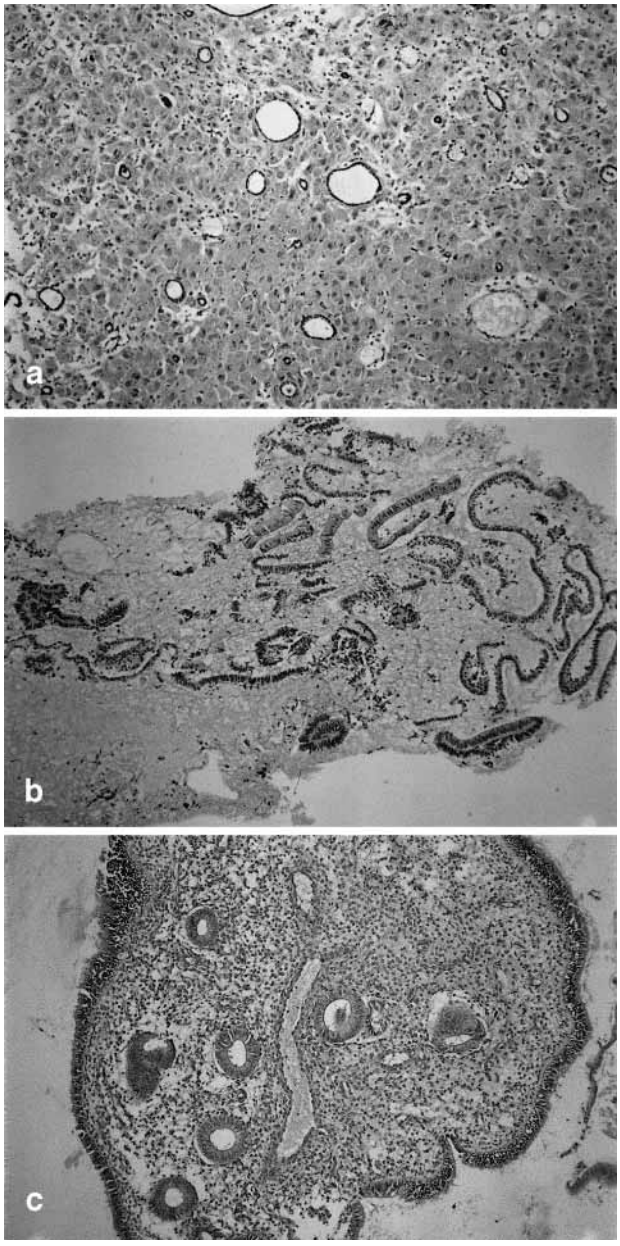

Fig. 1. Panel 1A: Endometrial sample showing epithelial atrophy with decidualization of the stroma typical in the LNG-IUD group.
Panel 1B: Inactive endometrium with no mitoses in epithelial cells, where the epithelium is single-layered. Seen in both the LNG-IUD and the progesterone groups
Panel 1C: Estrogen effects dominating in the endometrium seen as proliferative changes: mitoses in epithelial cells, with the epithelium pseudostratified.

The endometrial morphologies during oral and vaginal administration of natural progesterone were comparable. Roughly half of the specimens showed proliferative features, and only four out of 19 endometria in the oral P and five out of 14 in the vaginal P group were inactive (Table II).

Two samples in the oral P group presented class II changes according to the Papanicolaou classification at the end of the trial. One of them had bleeding and the endometrium was 6.6 mm thick, but the other had no bleeding problems and the endometrial thickness was only 2.7 mm. The histological specimen showed no hyperplasia, while a marked estrogen effect on the endometrium could be seen in both cases.

There was no clinical endometritis in any of the women. One endometrial sample of a woman without any symptoms of endometritis showed plasma cells, possibly as a sign of a foreign body reaction.

Discussion

Endometrial thickness up to 5 mm has been considered normal in postmenopausal women (14). In our study it remained clearly below this value, and there were only six patients with an endometrium thicker than 5 mm. The LNG-IUD did not disturb the measurement of endometrial thickness, because its echo is not as strong as that of a copper device due to the absence of metal. This explains why the endometrium of the women with a LNG-IUD was not thicker than that of the other women. The present forms of HRT did not affect uterine size, which is in accordance with the previous results (15).

Ultrasonographic examination of the pelvis is a valuable tool for detecting abnormalities of the postmenopausal endometrium, and it also gives information about the myometrium and the ovaries. It has been claimed that ultrasound is better than a cytological/histological evaluation of endometrial samples for the follow-up of postmenopausal endometrium during HRT (16). In our opinion, these methods complement each other without any preference. The finding that there was no difference in endometrial thickness between the groups, while the morphological changes between the LNG-IUD group compared to the oral and vaginal P groups were strikingly different, support our view. We therefore agree with the concept that endometrial sampling is necessary in women using HRT especially if there is break-through bleeding (17).

We have used three criteria for a desirable endometrial response to progestin in long-term combined HRT. Firstly, the absence of mitoses in epithelial cells is a sign of an inactive endometrium. Secondly, stromal reaction with decidualization is an even more powerful indicator of the response of an estrogen-primed endometrium to progestin. Thirdly, insulin-like growth factor binding protein-1 (IGFBP-1) on endometrial stromal cells is also a marker of such a response. IGFBP-1 is normally found in endometrial stromal cells during the secretory phase of the menstrual cycle and during pregnancy. Two studies (12, 18) have confirmed its presence in the endometrium of estrogen-treated postmenopausal women using LNG-IUD. It indicates effective suppression and counter-action of LNG to the stimulatory effects of estrogen and protection against hyperplasia and malignant transformation of the endometrium, as mRNA encoding IGFBP-1 is lacking in endometrial cancer (19). In addition, clinical conditions known to increase the risk of endometrial cancer are characterized by decreased or inhibited production of endometrial IGFBP-1 (19) while mRNAs encoding IGFs and IGF receptors stimulated by estrogen are also expressed in the endometrium of postmenopausal women who use no estrogen (20). At least two, and in most cases even three, of the above mentioned signs were detected in the endometrium of the women using LNG-IUD.

The IUD releases the progestin straight into the uterus, leading to a high endometrial concentration of progestin and minimizing systemic side-effects (21). Although persistent atrophy of endometrial epithelium has not been achieved by any form of HRT (22), the present findings suggest that LNG-IUD provides a good alternative method even in this respect to women who do not wish to have regular menstrual-like bleedings.

In our study, 100 mg of natural progestrone daily during 25 days per month did not induce a sufficient response to progestin to protect the endometrium against the proliferative effects of estrogen. Of the present markers, IGFBP-1 and epithelial atrophy together with stromal decidualization were absent in all women in the two natural progesterone groups (12). The bleeding profiles of the women in the progesterone groups were also poorer than those in the LNG group (8). Our histopathological and clinical findings are thus in marked contrast to the observations of Faguer et al. and Gillet et al. (9, 10), who recommended this treatment modality for clinical use.

The half-life of natural progestrone in serum is only 5 minutes, and the maximal serum concentration is seen four hours after oral and three to six hours after vaginal administration of natural progestrone (23). In both progesterone groups, a higher daily dose of natural progesterone, possibly divided into two doses, would be better. A HRT regimen with sequentially administered vaginal progesterone has been found to induce secretory

(24) or antiproliferative changes (25). Some studies have suggested that vaginal administration of natural progesterone would lead to more marked endometrial effects than oral progesterone (26, 27). However, our study showed that the endometrial responses to vaginal and oral progesterone were quite similar, even though some women doubled the dose of vaginal progesterone during the trial. There may be differences in the absorption of vaginally administered natural progesterone, depending on the vehicle of the suppository (28). The preparation used in our study induced good plasma levels of progesterone after vaginal administration in fertile-aged women (29). The vaginal anatomy of postmenopausal women is different from that of fertile-aged women due to relaxation of the pelvic floor. That may be one reason why the material of the suppository tended to come out of the vagina when it melted and we therefore did not see a sufficient endometrial response in most of the women in the vaginal progesterone group. It is also worth pointing out that the morphological state of the endometrium may not be uniform throughout the endometrium. This was also particularly true of the women in the progesterone groups, who had concomitantly both inactive and proliferative areas in their endometrium.

In conclusion, LNG-IUD induces excellent suppression of the endometrium whereas a daily dose of 100 mg of oral and 100–200 mg of vaginal natural progesterone for 1–25 calendar days is too low to produce an adequate progestin effect in long-term use.

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