Micronized progesterone and its impact on the endometrium and breast vs. progestogens

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Key words: BREAST CANCER, ENDOMETRIAL CANCER, HORMONE REPLACEMENT THERAPY, MEDROXYPROGESTERONE ACETATE, MPA, PROGESTOGEN, PROGESTERONE

ABSTRACT

It is well established that progestogens protect the endometrium against the proliferative effects of estrogens in postmenopausal women receiving hormone replacement therapy (HRT). Therefore, micronized progesterone and progestogens are recommended as part of combined HRT in women with an intact uterus. The protective effect of progestogens against hyperplasia and endometrial cancer does not appear to differ with different progestogens (micronized progesterone or progestogens), but appears to be affected by the regimen and thus the dose, with continuous combined treatment conferring better protection. However, the protective effect of progestogens seen in the endometrium is not replicated in the breast. Progestogens combined with estrogens are generally associated with a small increase in the risk of invasive breast cancer, which is believed to be due to a promoter effect. However, all progestogens are not equivalent in their effects on the breast and breast cancer risk. Micronized progesterone does not increase cell proliferation in breast tissue in postmenopausal women compared with synthetic medroxyprogesterone acetate (MPA). Experimental evidence suggests that the opposing effects of MPA and micronized progesterone on breast tissue are related to the non-specific effects of MPA, including glucocorticoid activity and differences in the regulation of gene expression. Therefore, for women with an intact uterus, micronized progesterone may be the optimal choice as part of combined HRT.

INTRODUCTION

It is now well established that unopposed estrogen as hormone replacement therapy (HRT) in postmenopausal women induces a dose-related stimulation of the endometrium associated with an increased risk of hyperplasia and endometrial cancer. As micronized progesterone and progestogens protect the endometrium against the proliferative effects of estrogens, women with a uterus should receive combined HRT with estrogens and micronized progesterone or progestogens. Unfortunately, the protective effect of progestogens seen in the endometrium is not replicated in the breast.

HRT AND THE ENDOMETRIUM

Micronized progesterone and progestogens are indicated to prevent the proliferative effect of estrogens in women with a uterus. However, continuous low-dose or short-term sequential progestogen administration is not sufficient to alleviate the risk of endometrial cancer associated with estrogens. Recently, long cycles (progestogen for 10–14 days every 3 months) have also been shown to increase the risk of endometrial cancer.

Most studies demonstrate that continuous combined HRT does not increase the risk of endometrial cancer and may even reduce it, particularly in obese women. Continuous conjugated equine estrogens (CEE, 0.625 mg) plus medroxyprogesterone acetate (MPA, 2.5 mg) did not significantly affect the risk of endometrial cancer in 16,608 women in the randomized, double-blind, placebo-controlled Women’s Health Initiative (WHI) study who were followed up for 5.2 years, compared with placebo (hazard ratio (HR) 0.83; adjusted 95% confidence intervals (CI) 0.29–2.32). Moreover, continuous combined HRT significantly reduced the risk of endometrial cancer (relative risk (RR) 0.71; 95% CI 0.56–0.90; p = 0.005) in 69,577 women receiving it and followed up for...
3.4 years in the Million Women Study, compared with never-users (Table 1). Furthermore, the beneficial effects of continuous combined HRT increased with increasing obesity (p = 0.003). Overall, these data demonstrate that the risk of endometrial cancer does not differ with the progestogen used, but the regimen (continuous rather than sequential), and thus the dose, can affect the risk; the duration of progestogen use can also affect the risk of endometrial cancer.

A recent observational cohort study by the European Prospective Investigators into Cancer and Nutrition (EPIC) involving 115 474 postmenopausal women recruited from ten countries and followed up for 9 years indicated an increased risk of endometrial cancer in a small group of women receiving oral micronized progesterone (HR 2.42; 95% CI 1.53–3.83), but not in women receiving synthetic progestogens or testosterone derivatives.

**Weak plausibility of increased risk of endometrial cancer associated with micronized progesterone**

The conclusion that micronized progesterone was associated with an increased risk of endometrial cancer in the EPIC study was based on only 26 cases of endometrial cancer in 2231 women receiving micronized progesterone in this study, a small number, as acknowledged by the authors. In addition, HRT use was only noted in a single baseline assessment.

As in the Million Women Study, the risk of endometrial cancer was strongly associated with body mass index (BMI) in women receiving combination therapy in this study, with a significantly higher risk in women with normal BMI (<25 kg/m²; HR 1.49; 95% CI 1.05–2.13) than in overweight women (BMI 25–29 kg/m²; HR 1.24; 95% CI 0.74–2.07) and obese women (BMI ≥ 30 kg/m²; HR 1.29; 95% CI 0.65–2.55), compared with never-users. The vast majority of women receiving micronized progesterone were French, as demonstrated in the breast cancer cohort of the EPIC study (n = 133 744) in which 24% of women in France received micronized progesterone, along with 2% in Italy, and 1% in both Spain and The Netherlands. The French cohort in the EPIC study represents the E3N population. Women enrolled in the EPIC study in France had the lowest BMI in the predominant population receiving micronized progesterone could explain this result in the EPIC study, as demonstrated in the Million Women Study. Furthermore, no information regarding the sequence and dose of micronized progesterone was available for the French women receiving it in this study, and sequential therapy was gradually being replaced with continuous therapy during the follow-up of this study. In the meantime, the importance of the effect of sequential vs. continuous combined HRT on the endometrium was clearly demonstrated in a prospective study in postmenopausal women that showed that continuous combined HRT for 9 months converted the endometrium to normal in women who developed hyperplasia, including complex hyperplasia, whilst receiving sequential therapy. Progestogen, more commonly micronized progesterone, given on days 1–25 days followed by a break of 5 days is now the most commonly used regimen as part of combined HRT in France.

The results of at least two randomized clinical studies argue against a possible increase in endometrial cancer risk in association with micronized progesterone. The Postmenopausal Estrogen/Progestin Interventions (PEPI) study was a multicenter, randomized, double-blind, placebo-controlled study undertaken to determine the effects of unopposed estrogens (CEE 0.625 mg) and estrogens (CEE 0.625 mg) plus progestogens (continuous MPA 2.5 mg, sequential MPA 10 mg/day for 12 days, or micronized progesterone 200 mg/day for 12 days) compared with placebo on the histology of the endometrium of 596 postmenopausal women. After 3 years, there were no significant differences in the occurrence of abnormal biopsy specimens in women given micronized progesterone vs. placebo or MPA (continuous or sequential; Table 1). A second double-blind study randomized over 300 postmenopausal women to receive estradiol (1.5 mg on days 1–24) with either chloroacinnone acetate (CA) 10 mg or micronized progesterone 200 mg daily on days 11–24 to determine their comparative effects on endometrial histology. At baseline, 92% of women (247/269) had an atrophic endometrium and 15% (18/115) were not evaluable as insufficient biopsy material was obtained, mainly due to the endometrium being too atrophic. A further 42 women receiving CA and 72 receiving micronized progesterone were also not evaluable due to insufficient biopsy samples at 18 months (Table 2). Biopsy results at 18 months demonstrated atrophy in 20% and 27% of women receiving CA and micronized progesterone, respectively, with a very low proliferative rate in both cohorts (Table 2). If one considers that, of those samples that were not evaluable, the majority were not evaluable due to atrophy, the incidences of atrophy in women receiving CA and micronized progesterone were 53% and 87%, respectively, clearly demonstrating that micronized progesterone is not proliferative in the endometrium, but has the opposite action.

Another reasonable explanation for the possible increased risk of endometrial cancer observed with micronized progesterone in the EPIC study is that compliance with oral progesterone was low. The HRT regimen combining transdermal estradiol with oral micronized progesterone could result in lower compliance, as synthetic progestogens are available as fixed combinations with estradiol. These results suggest that compliance should be systematically monitored during the consultation, and that the reasons underlying the need for the progestogen component of HRT should be explained to the patient in detail.
HRT AND THE BREAST

Effect of endogenous sex hormones in normal breast cells

Various studies have described the morphological and histological changes in normal human breast tissue associated with the menstrual cycle, but this topic has been the subject of much debate.

Vogel and colleagues characterized the morphological changes in the breast during the menstrual cycle into five phases: the proliferative phase (days 3–7), the follicular phase (days 8–14), the luteal phase of differentiation (days 15–20), the secretory phase (days 21–27), and the menstrual phase (days 28–2), using biopsy data from premenopausal women undergoing subcutaneous mastectomy or reduction mammoplasty (without neoplasia). A few years later, it was shown that the peak of proliferation was in fact in the latter half of the cycle when progesterone reaches maximal concentrations, rather than in the first half of the cycle when estrogens reach peak levels, as proposed by Vogel and colleagues. In one study, proliferation was assessed using a thymidine labelling index and samples obtained from women undergoing biopsy or mastectomy for benign or cancerous conditions. However, the hormone environment was not evaluated and therefore accurate determination of the stage within the menstrual cycle was not possible in this study. A study using autopsy samples allowed the correlation of breast and endometrial morphology. This study also showed that peak proliferation occurred near the end of the cycle in the late luteal phase (days 23–25). This finding was later confirmed using samples from women undergoing surgery for benign breast disease for whom accurate data concerning the day of the menstrual cycle on which the biopsy was taken were available. Not only did this latter study confirm that the peak of proliferative activity occurred in the late luteal phase of the cycle and was, in general, very low in the normal breast, it also demonstrated that increased proliferation during this stage was generally associated with an increase in apoptosis. Does this increase in apoptosis compensate for the peak in proliferation?

Risk of breast cancer with HRT

Most published epidemiological studies, but not all, indicate a small increased risk of invasive breast cancer with long-term combined HRT including a synthetic progesterogen, with a lower risk in women receiving unopposed estrogen. In the WHI study, continuous combined HRT (CEE 0.625 mg plus MPA 2.5 mg) was associated with a non-significant trend towards an increased risk of breast cancer (HR 1.26; adjusted 95% CI 0.83–1.92), compared with non-users after a mean follow-up of 5.2 years. In the estrogen-only part of the WHI study, CEE alone was associated with a reduced risk of breast cancer (HR 0.77; adjusted 95% CI 0.57–1.06) after an average follow-up of 6.8 years. In the Million Women Study, there were 9364 cases of invasive breast cancer and 637 associated deaths after an average follow-up of 2.6 and 4.1 years, respectively, in 1084110 UK women aged 50–64 years, half of whom used HRT at

Table 1 Endometrial changes in women receiving unopposed estrogens (conjugated equine estrogens (CEE)) or combination therapy (medroxyprogesterone acetate (MPA) or micronized progesterone (MP)) in the PEPI study (follow-up 3 years). Reproduced with permission from The Writing Group of the PEPI Trial.

<table>
<thead>
<tr>
<th>Endometrial biopsy changes (n)</th>
<th>Placebo (n = 119)</th>
<th>Unopposed CEE (n = 119)</th>
<th>CEE + MPA (sequential) (n = 118)</th>
<th>CEE + MPA (continuous) (n = 120)</th>
<th>CEE + MP (sequential) (n = 120)</th>
<th>Total (n = 596)</th>
</tr>
</thead>
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<td>45</td>
<td>112</td>
<td>119</td>
<td>114</td>
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<td>33†</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>44 (7%)</td>
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<tr>
<td>Complex (adenomatous) hyperplasia</td>
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<td>27†</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Atypia</td>
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<td>14†</td>
<td>0</td>
<td>0</td>
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<td>15 (2%)</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (&lt;1%)</td>
</tr>
</tbody>
</table>

*, p = 0.16 (normal vs. abnormal) vs. placebo; †, p < 0.001 vs. placebo

Table 2 Endometrial changes at 18 months in postmenopausal women receiving estradiol plus chloroaminone acetate or micronized progesterone. Data are given as number of women (%). Reproduced with permission from Jondet et al.
some time. In current HRT users, the combination of estrogens and synthetic progestogens doubled the risk of breast cancer (adjusted RR 2.0; 95% CI 1.88–2.12; *p* < 0.0001), and was associated with a higher risk of invasive breast cancer than other HRT regimens (unopposed estrogen and tibolone; *p* < 0.0001).

However, data also suggest that progestogens are not equivalent in terms of the risk of breast cancer associated with their use, as micronized progesterone and dydrogesterone do not appear to increase the risk of breast cancer in comparison with their use, as micronized progesterone and dydrogesterone (unopposed estrogen plus progestogens adjusted within 3 years of menopause; HR 1.89; 95% CI 1.53–2.34), but not with dydrogesterone (HR 1.38; 95% CI 0.98–1.93). These fluctuations in risk may suggest some confounding bias or the low power of the study due to the low number of breast cancer cases.

The general consensus is that HRT exerts a promoter effect, i.e., HRT promotes an existing breast tumor rather than initiating one, as seen with oral contraceptives. This consensus is based on the decreased risk of breast cancer observed after the discontinuation of HRT.

**Effect of exogenous progestogens on normal breast cells**

Three intervention trials have investigated the effect of exogenous progestogen and...
progesterogens (and estrogens) on the proliferation of normal breast cells. In the first randomized, double-blind, placebo-controlled trial, premenopausal women undergoing breast surgery for the removal of a lump applied topical estradiol and/or progesterone to the breast for 11–13 days prior to surgery. In agreement with the known effects of estradiol on breast cells, estradiol significantly increased cell proliferation in breast tissue, assessed by measuring mitosis or using proliferating cell nuclear antigen (PCNA), a cell cycle marker \( (p < 0.05 \text{ vs. placebo}) \). Progesterone alone significantly reduced cell proliferation \( (p < 0.05 \text{ vs. placebo}) \), and reversed the proliferative effect of estradiol to the levels achieved with placebo (mitosis) or below (PCNA; \( p < 0.05 \text{ vs. placebo}) \). The reversal of the proliferative effect of estradiol by progesterone was also seen in a similar study undertaken using breast tissue obtained from postmenopausal women undergoing cosmetic breast surgery or removal of a breast lump.

The final study randomized postmenopausal women to receive continuous CEE (0.625 mg) with MPA added for 14 of 28 days per cycle, or transdermal estradiol (1.5 mg) with oral micronized progesterone for 14 of 28 days per cycle, given for two cycles. Biopsies were taken before and after treatment; cell proliferation was measured using Ki-67/MIB nuclear antigen expression and apoptosis was measured using immunostaining with the antiapoptotic protein bcl-2. CEE plus MPA significantly increased cell proliferation compared with baseline \( (p = 0.003) \) and compared with transdermal estradiol plus micronized progesterone \( (p = 0.05) \); Figure 2. Transdermal estradiol plus micronized progesterone did not significantly increase cell proliferation compared with baseline. Both HRT regimens showed a trend towards reduced apoptosis \( (p = 0.06 \text{ for transdermal estradiol plus micronized progesterone}) \).

These data reflect experimental data in cynomolgus monkeys in whom menopause was induced by ovariectomy and which also demonstrated that different progesterogens have different effects on breast cells. In this monkey model, estradiol plus progesterone did not significantly increase proliferation in breast epithelial cells \( (p = 0.47 \text{ for lobular cells and } p = 0.72 \text{ for ductal cells vs. placebo}) \), measured using Ki-67 expression. In contrast, estradiol plus MPA significantly increased breast cell proliferation; lobular proliferation was increased by 194\% \( (p = 0.009 \text{ vs. placebo}) \) and ductal proliferation by 544\% \( (p = 0.006 \text{ vs. placebo}) \). Neither HRT regimen had a significant effect on apoptosis and cell survival. Further investigation into the effects of different progesterogens on gene expression in the breast of cynomolgus monkeys demonstrated that the expression of genes related to epidermal growth factor (EGFR) activity were higher after treatment with estradiol plus MPA, compared with estradiol plus progesterone, suggesting that the proliferative effects of MPA may be mediated, at least in part, by increased EGFR activity.

A study in normal luminal human breast cells in vitro further demonstrated that the regulation of gene expression was significantly different in response to estradiol plus progesterone compared with estradiol plus MPA (Figure 3). Gene ontology analysis demonstrated that estradiol alone highly enriched gene families associated with cell death \( (p = 0.00002) \) and cell growth and proliferation \( (p = 0.0012) \); estradiol plus progesterone significantly enriched genes associated with cell morphology and cellular function and maintenance \( (p = 0.00036 \text{ for each}) \); and estradiol plus MPA significantly enriched genes associated with cell death and small molecular biochemistry \( (p = 0.0006 \text{ for each}) \). Indeed, only 39 genes were commonly regulated by progesterone and MPA (plus estradiol; Figure 3).

Potential mechanisms underlying the effect of MPA in breast cells: non-specificity

In this in vitro study, we investigated the tumorigenic potential of progesterone and MPA (with estradiol) and determined whether their opposing effects on cell proliferation in normal human breast cells were due to their different steroidal properties. We confirmed that progesterone counteracted the proliferative effect of estradiol in normal human breast cells. MPA also counteracted the effect of estradiol but to a much lower extent in these cells. In breast cancer cells, progesterone counteracted the proliferative effect of estradiol in T47-D cells, but not in MCF-7 cells. However, in contrast to normal human breast cells, MPA reduced cellular proliferation in response to estradiol in both MCF-7 and T47-D breast cancer cells. We demonstrated that T47-D breast cancer cells lack glucocorticoid receptors (GR), whereas MCF-7 cells and normal breast cells

![Figure 2](image-url) Comparative breast cell proliferation effects of oral conjugated equine estrogens (CEE) with medroxyprogesterone acetate (MPA) and transdermal estradiol (E2) plus oral micronized progesterone in the breast cells of healthy menopausal women \( (n = 71) \). Adapted from Murkes et al. NS, not significant; HRT, hormone replacement therapy.
Impact of micronized progesterone on endometrium and breast
gompel

Climacteric 23

...cells contained a high level of GR. Furthermore, in the MCF-7 cell clone used, progesterone receptors seemed to be non-functional. A pure, potent glucocorticoid (dexamethasone) was mitogenic and antiapoptotic in normal human breast cells and antiproliferative and antiapoptotic in breast cancer cells. MPA was shown to activate GR in both normal human breast cells and MCF-7 breast cancer cells, using a glucocorticoid antagonist and the down-regulation of the glucocorticoid receptor by RNA interference. MPA is known to be non-selective and have significant glucocorticoid activity, whereas progesterone has no glucocorticoid activity. Given the similar effects of progesterone and MPA in T47-D breast cancer cells (devoid of GR) and their differential effects in normal human breast and MCF-7 cells, these data suggested that the effect of MPA on proliferation may be in part driven by its glucocorticoid activity.

Potential mechanisms underlying the effect of progesterone in breast cells

Recent data suggest that progesterone (plus estradiol) could be a promoter of luminal progenitor cell proliferation in the mammary glands of mice and human breast cells in vitro. Progesterone could increase the proliferation of luminal progenitors by an indirect mechanism through the induction of the receptor activator of NF-κB ligand (RANKL). The RANKL receptor RANK is present in the progenitor cells, which are devoid of progesterone receptors, and thus the RANKL/RANK system could control their proliferation by a paracrine effect. It was also reported that the incidence and onset of MPA-driven breast cancer in mice were associated with a massive induction of RANKL in mammary gland epithelial cells; inactivation of RANK in these cells prevented MPA-induced epithelial proliferation, impaired expansion of stem cells, and sensitized the cells to DNA damage-induced cell death. However, no comparison between the potencies of MPA and progesterone was provided. These observations, mostly reported in murine models, remain to be confirmed in humans.

CONCLUSIONS

Micronized progesterone and progestogens as part of combined HRT do not have equivalent effects in the breast. Micronized progesterone, the natural progestogen, appears to have a more favorable effect on breast tissue than synthetic progestogens such as MPA, possibly due to its high selectivity and lack of glucocorticoid activity. Despite the results of the EPIC study, the majority of data suggest that progesterone and progestogens do not differ with respect to their protective effect on the endometrium. Based on these data, progesterone may be the optimal progestogen...
for use as part of combined therapy in women with an intact uterus.

**ACKNOWLEDGEMENT**

Assistance with writing this manuscript was provided by Clare Ryles, medical writer, and funded by Besins Healthcare.

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