

## Invited Editorial

# Effects of progestogens on the postmenopausal breast

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## ABSTRACT

The potential for an increased risk of breast cancer linked to the use of synthetic progestins combined with oral estrogens is one of the main putative reasons for discouraging postmenopausal women from using any type of hormone replacement therapy (HRT) for more than a few years. Because no definitive proof exists, the available epidemiological results can be interpreted according to what seems biologically plausible to each investigator, including potential differences between various schedules of various steroids in various species and *in vitro* models.

More than 60 years after the discovery of progesterone, the main effects of this endogenous steroid on the physiopathology of the breast during a normal luteal phase are still controversial. The lack of consensus on such basic knowledge concerning one of the most important targets of a natural ovarian hormone discovered in 1934 is amazing. In the most cited studies, nothing has been done to measure progesterone in plasma and to correlate the extremely disparate cytological results with extremely erratic steroid levels at the time of surgical stress. In a recent study, with a better design, the physiological rise of endogenous progesterone during the luteal phase coincided with a drop in proliferation of breast epithelial cells, which appears to be only slightly delayed in comparison with what is described in the endometrium. Differences in doses and schedules of treatments with various synthetic progestins have largely contributed to the inconsistency in clinical recommendations. Based on the analysis of proliferation markers in surgical biopsies from normal human postmenopausal breast tissue, it is plausible that mitogenic activity is not identical during therapy with unopposed estrogens versus estrogens combined with progestogens, and is higher during HRT that combines oral conjugated equine estrogens with medroxyprogesterone acetate than during HRT that combines transdermal estradiol and progesterone. It is misleading to put all progestogens in the same bag irrespective of their chemical structure, and, more important, their effect may vary according to whether it is estrone or estradiol that is mainly accumulated in the breast tissue. The hypothesis of progesterone decreasing the proliferative effect of estradiol in the postmenopausal breast remains highly plausible.

Breast cancer incidence increases with age and most breast cancers are diagnosed after the menopause<sup>1</sup> when serum estradiol and progester-

one levels are very low, but a major role of these hormones in the development of the disease is suggested by several observations.

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First, a slowing of the rate of increase in breast cancer incidence around age 50 years is generally presented as evidence of a dependence on serum levels of ovarian hormones. However, this slowed rate of increase affects only the recorded incidence of breast cancer diagnosis and not the breast cancer mortality. This may be partly due to the decrease in regular mammography screening observed after the menopause in untreated women<sup>2</sup>. When changes occur in the way that breast cancer is diagnosed, trends in recorded incidence rates should be interpreted with caution<sup>3</sup>. If, independently of mammography screening, early menopause actually reduces the breast cancer risk, and late age at menopause increases it<sup>4</sup>, then the ovarian secretion of estradiol during the perimenopausal years should play an especially important role at early stages of the disease. Because the incidence of anovulatory cycles increases during these perimenopausal years<sup>5</sup>, the endogenous secretion of progesterone can only be negatively related to the increase of breast cancer risk linked to late menopause.

Second, in untreated postmenopausal women, the highest residual serum estradiol (and testosterone) levels are associated with a higher risk of breast cancer<sup>6–8</sup>. However, the serum estradiol threshold separating the high- and low-risk groups after the menopause is very low, i.e. 3–10 pg/ml for total estradiol<sup>6,7</sup> and 2 pg/ml for bioavailable estradiol<sup>8</sup>, suggesting that the circulating estrogens become far less important than the local synthesis in breast cancer cells. Breast adipose tissue and most carcinomas acquire aromatase and 17 $\beta$ -hydroxysteroid dehydrogenase activities efficient enough to synthesize estradiol locally from androgenic substrates, which is a pivotal mechanism in maintaining the growth of estrogen-dependent cancers, independently of serum estrogen levels<sup>9</sup>. The very small difference between the highest and lowest postmenopausal serum estradiol levels may simply reflect the level of aromatase activity in several tissues, but not an actual direct dependence of breast cancer cells on circulating levels of estrogens.

Therefore, the overall risk, if any<sup>9,10</sup>, of again raising serum estrogens in postmenopausal women is low<sup>4</sup>. It may only be relevant to women with a lean body weight, likely to exhibit low aromatase activities. If these women use estrogen replacement therapy, their breast cancer risk increases to the level observed in untreated postmenopausal women who are slightly overweight. This risk is not dose-related, and involves

mostly small, well-differentiated, lymph node-negative breast cancers. This may create early detection bias in treated women who are more frequently screened by mammograms. The use of an estrogen replacement therapy does not alter the breast cancer risk in women who are slightly overweight and therefore likely to show relatively high aromatase activities.

Because progesterone secretion decreases during the pivotal perimenopausal years<sup>5</sup> and no local synthesis of progesterone occurs after the menopause, this endogenous hormone should not have a major influence on postmenopausal breast cancer. However, despite almost 100 epidemiological studies analyzing the risk of cancer during hormone replacement therapy (HRT), the specific influence of exogenous progestogens administered in combination with estrogens is still the subject of debate<sup>4,10–19</sup>.

## EPIDEMIOLOGICAL STUDIES

In most studies, combined HRT users still account for a small minority of the cases, selected according to unspecified and probably variable criteria, while the majority have used unopposed estrogens. This selection, unrestricted to non-hysterectomized women, weakens the power of statistical analysis and introduces potential biases<sup>11</sup>. Only 4–12% of the treated populations included in US cohorts in the 1970s, 1980s and 1990s used a progestin combined with estrogens, while the majority received unopposed estrogens<sup>4</sup>. For example, among 2082 postmenopausal breast cancer patients analyzed in a recent US cohort study<sup>13</sup>, only 101 (4.8%) used a progestin, which was added for about 10 days per month to an estrogen. The overall relative risk (RR) of breast cancer (1.3, confidence interval (CI) 1.0–1.6) was not significantly increased in this subgroup. However, a harmful effect associated with the duration of estrogen–progestin use was reported, based on only 18 cases (0.8%) of invasive breast cancer diagnosed in recent users of combined HRT for  $\geq 4$  years with a body mass index of  $\leq 24.4$  kg/m<sup>2</sup>. In this subgroup the RR was 1.9 (CI 1.1–3.3) overall, and 1.08 per year of use (CI 1.02–1.16).

Multiplying small, selected subgroups increases the risk of results being simply due to chance distribution of collected or uncollected subject characteristics, and hence may be misleading<sup>11</sup>.

In one study, only the sequential use of a progesterone-derived progestin (5.3% of cases) appeared to be associated with a significantly

increased risk<sup>13</sup>. Another study showed that only the continuous combined use of testosterone-derived progestins (1.9% of cases) was associated with an increased risk<sup>16</sup>, and in a third the risk was similar for the use of oral estrogens alone and for sequential or continuous combined HRT (11.2% of cases)<sup>19</sup>.

No definitive proof exists of a potential influence of progesterone, and the available epidemiological results can be interpreted according to what seems biologically plausible to each investigator<sup>17,18</sup>, including potential differences between various regimens, in various species and in various *in vitro* models<sup>12</sup>.

When seeking biological plausibility, the pivotal debate still concerns the effects of natural progesterone on human breast epithelial cell proliferation. The lack of consensus on such basic knowledge concerning one of the most important targets of a natural ovarian hormone discovered<sup>20</sup> more than 60 years ago (1934) is amazing.

## PROGESTERONE AND HUMAN BREAST EPITHELIAL CELL PROLIFERATION *IN VITRO*

Because of its rapid metabolization, progesterone has rarely been used in *in vitro* studies, and even in recent studies it is frequently replaced by various synthetic progestins<sup>21,22</sup> with longer half lives but potentially different effects. In the few *in vitro* studies using natural progesterone, it down-regulates estradiol receptors and inhibits the cell proliferation stimulated by estradiol in normal human breast epithelial cells<sup>23</sup>. Similarly, progesterone arrests human breast cancer cells in the G<sub>1</sub> phase of the second cycle by up-regulating cyclin-dependent kinase inhibitors (p21 and p27) and down-regulating cyclin D<sub>1</sub><sup>24</sup>. Some studies performed on breast tumor cell lines show a pro-apoptotic effect of progesterone<sup>25</sup>, reproduced by a pregnane-progestin<sup>21</sup>, while another study shows an anti-apoptotic effect of medroxyprogesterone acetate (MPA)<sup>26</sup>. Therefore, *in vitro* studies suggest potential benefits from progesterone in regulating human breast epithelial cell growth. However, because large discrepancies appear between various synthetic progestins<sup>26,27</sup> and antiprogestins at various concentrations in various milieux, these results are still poorly predictive of what actually happens in the human breast *in vivo*<sup>12</sup>. Probably because of their increasing complexity, and despite their use of objective and reproducible measurements, these *in vitro*

studies have not yet delivered a message consistent and clear enough to help clinicians.

## ENDOGENOUS PROGESTERONE AND HUMAN BREAST EPITHELIAL CELL PROLIFERATION *IN VIVO*

In agreement with *in vitro* experiments<sup>23</sup>, the endogenous surge of progesterone during the luteal phase induces a down-regulation of estrogen receptors in normal breast epithelial cells<sup>28</sup>. It thus seems reasonable to expect some anti-estrogenic effects from progesterone. As progesterone does not down-regulate its own receptors in breast cells, its effects here may differ from those well known in the endometrium<sup>28</sup>. Many papers report, as an established fact, that progesterone is the major mitogen in the normal breast epithelium of premenopausal women<sup>12,15,17,18,29</sup>. The studies most often cited to support this statement analyzed the proliferative activity in surgical breast biopsies collected on different days of the menstrual cycle<sup>30,31</sup>. Because the highest values of thymidine index were measured in some of the women having surgery around days 21–25, a mid-luteal rise in progesterone was supposed to be the main mitogenic factor. However, a considerable intersubject variability in thymidine index was observed during the same days of the luteal phase. This variability was far beyond what could be expected from technical problems<sup>28</sup>, but no attempt was made to explain why between day 21 and day 25 some patients showed high mitotic activity and others low. Specifically, no attempt was made to correlate thymidine index with either estradiol or progesterone serum or tissue levels, which were not measured.

However, probably due to stress anovulation, one study measured very low serum and breast tissue progesterone levels in 40% of premenopausal patients when surgery was done during the expected luteal phase<sup>32</sup>. Therefore, from these first studies, it is impossible to know whether women with a high mitotic activity during the second part of their menstrual cycle are ovulatory women with high progesterone levels or, conversely, anovulatory women with low progesterone levels.

Attempts to avoid surgery by using fine-needle aspirations in one recent study<sup>33</sup> may have been misleading<sup>34</sup>, because aspirations produce only hundreds of epithelial cells while surgical biopsies produce the thousands required for sufficient statistical power. Also, in the same study, the use of Ki-67 to replace the relatively imprecise thymidine index was inappropriate. This marker

of the cell cycle has a short half-life of about 1 h, and produces a signal too weak to identify variation when the average mitotic activity is low, as is seen in normal breast epithelial cells. Ki-67 labelling is detected in < 1% of normal breast epithelial cells in treated and untreated postmenopausal women, which is not enough to detect a significant change during estrogen replacement in postmenopausal women<sup>35,36</sup>, while proliferating cell nuclear antigen (PCNA), with a longer half-life of about 20 h, labels 8–20% of these cells and shows a significant increase in proliferation during estrogen therapy<sup>36–38</sup>.

Only one study, excluding anovulatory women, has measured plasma and tissue progesterone concentrations, the epithelial cell cycle using PCNA and apoptosis (terminal uridine deoxynucleotidyl nick end labelling, TUNEL) in surgical material obtained after mastoplasty<sup>39</sup>. With this improved design, the epithelial cell growth was shown to be directly related to the estradiol/progesterone tissue ratio. This was significantly lower during the luteal than the follicular phase. In comparison with what is known for the endometrium, the slowing of the epithelial cell cycle during the endogenous progesterone surge was slightly delayed and of less magnitude in the breast.

## EXOGENOUS PROGESTERONE AND HUMAN BREAST EPITHELIAL CELL PROLIFERATION *IN VIVO*

Few studies have tried to investigate the effects of natural progesterone administration on normal human breast *in vivo*. Two have compared the effects of topical applications on the breast skin of gels containing either a placebo, 17 $\beta$ -estradiol or progesterone during the follicular phase of premenopausal women scheduled for breast surgery<sup>32,37</sup>. A third study conducted the same experiment in postmenopausal women<sup>38</sup>. These regimens produced different concentrations of estradiol and/or progesterone within the breast tissue of normal women *in vivo*. In these three studies, high estradiol and low progesterone tissue levels were associated with the highest mitotic activity, measured either by mitotic figure counting<sup>32</sup> or by PCNA labelling<sup>37,38</sup>. Tissue concentrations of progesterone similar to those measured during a normal luteal phase were constantly associated with a lower rate of mitotic activity. These studies were consistent in showing that natural progesterone alone does not stimulate epithelial proliferation, but opposes, in the short

term, the proliferative effects of natural 17 $\beta$ -estradiol. In comparison with placebo treatment, 14 days of transdermal estradiol treatment significantly increased the proliferation of normal breast epithelial cells but a combination of transdermal estradiol and progesterone did not, in both pre- and postmenopausal women.

## EXOGENOUS PROGESTINS AND HUMAN BREAST EPITHELIAL CELL PROLIFERATION *IN VIVO*

It is plausible that various combinations of estrogens and progestogens may differently influence the proliferation of human breast epithelial cells and the relative risk of breast cancer.

To date, the most widely studied estrogens are oral conjugated equine estrogens (CEE) and estradiol. The serum and breast tissue levels of estrone and estrone sulfate and the estrone/estradiol ratio are far higher following oral CEE or estradiol than during physiological ovarian activity or during transdermal estradiol treatment<sup>40–42</sup>. The first consequence is that the daily urinary excretion of 16OH-estrone, a metabolite classified as genotoxic<sup>43</sup>, has been shown to be abnormally high in users of oral estrogens<sup>44</sup>. Another consequence of high levels of estrone may be a difference in progestogen-stimulated 17 $\beta$ -hydroxysteroid dehydrogenase activities<sup>45</sup>. The balance between the two 17 $\beta$ -hydroxysteroid dehydrogenase isoforms, the first reducing estrone to estradiol and increasing estrogenic activity, and the second oxidizing estradiol to estrone and reducing estrogenic activity, may favor the synthesis of estradiol if the estrone/estradiol ratio is high in the epithelial cell environment. Then, the consequence of 17 $\beta$ -hydroxysteroid dehydrogenase activity stimulation by the progestin could be reversed if the main estrogen accumulated in breast tissue is estrone instead of estradiol, which may be crucial for breast cancer cell proliferation<sup>9</sup>.

In most epidemiological studies, CEE have been used occasionally in combination with MPA<sup>4</sup>, a synthetic progestin which may be different from progesterone in its effects on breast tissue. For example, in breast cancer cell lines, progesterone has been shown to induce apoptosis and reduce proliferation in some studies<sup>25</sup>, while MPA has been shown to do the reverse in others<sup>26</sup>. Also, MPA<sup>45</sup>, in contrast with progesterone<sup>46,47</sup>, has been described to stimulate the reductive 17 $\beta$ -hydroxysteroid dehydrogenase isoform more than the oxidative 17 $\beta$ -hydroxysteroid dehydrogenase isoform, in human breast cells.

According to surgical breast biopsies conducted in postmenopausal women, the epithelial mitogenic activity, measured by PCNA labelling, increases similarly during treatment with either oral CEE<sup>36</sup> or transdermal estradiol<sup>38</sup>. Activity increases even more so with HRT regimens combining oral CEE and MPA<sup>36</sup>, while the addition of progesterone to transdermal estradiol decreases it<sup>38</sup>. These biological results, supported by some of the epidemiological surveys<sup>12–15</sup>, suggest that the HRT regimens combining oral CEE and MPA are not optimal for breast tissue and should be stopped after a few years of use<sup>17,18</sup>. Clear differences in the effects of a progesterone-derived progestin such as MPA and of a testosterone-derived progestin such as norethisterone or norgestrel have not been consistently established. The possibility that the binding of progestins to

the androgen or estrogen receptors, rather than the progesterone receptors, may simply predict their effects on human breast tissue *in vivo* has not been confirmed<sup>12,27,36</sup>. The potentially opposite effects of progestogens on human breast cell proliferation *in vivo* may depend primarily on the structure and concentration of the main estrogen, estrone or estradiol, accumulated in the breast tissue. The hypothesis of progesterone decreasing the proliferative effect of estradiol in the postmenopausal breast remains highly plausible.

*Conflict of interest* The author has served as a consultant for Asta-Medica, Besins-International, Bristol Myers, Hoechst Roussel, Janssen-Ortho, Pharmacia Upjohn, Schering, Shiseido, Solvay and Wyeth-Ayerst.

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