

Review

Progesterone receptors – animal models and cell signalling in breast cancer Implications for breast cancer of inclusion of progestins in hormone replacement therapies

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Abstract

Progestins are included in menopausal hormone replacement therapy to counteract the increased risk for endometrial cancer associated with estrogen replacement therapy. Studies of hormone replacement therapy and breast cancer risk and of changes in mammographic density according to different regimens of hormone replacement therapy suggest that, for the most part, estrogen-progestin replacement therapy has a more adverse effect on breast cancer risk than does estrogen replacement therapy. Many questions remain unresolved, however, including risk associated with different regimens of estrogen-progestin replacement therapy, and whether the effects vary according to tumor characteristics, such as histology, extent of disease, and hormone receptor status.

Keywords: breast cancer risk, estrogen, hormone replacement therapy, progestin

Introduction

Menopausal hormone replacement therapy (HRT), most commonly including estrogens alone or in combination with progestins, is used to alleviate menopausal symptoms and to prevent osteoporosis. Progestins are prescribed to offset the increased risk for endometrial cancer associated with estrogen replacement therapy (ERT). In the USA, it has become increasingly common to prescribe estrogens in combination with progestins since the early 1980s. An estimated 45% of menopausal US women aged 25–74 years in the early 1970s reported ever using HRT. Of those reporting HRT use in 1992, 31% reported taking progestins [1]. Use of progestins began earlier in Scandinavian countries than in the USA [2].

Assessing breast cancer risk associated with HRT is complicated by the fact that many different hormones, regi-

mens, and routes of administration have been used. During the 1980s the most common type of estrogen-progestin replacement therapy (EPRT) in the USA consisted of estrogens administered for the first 21–25 days of the calendar month and progestins added cyclically during the last 10–14 days of estrogen treatment. Other regimens, including continuous daily treatment with both estrogens and progestins, were developed to avoid the withdrawal bleeding that many women experience with cyclic therapy [3]. More recently, new regimens were introduced to prevent or minimize the breakthrough bleeding that is common during the first months of combined/continuous EPRT. These include the use of progestins only every second or third month [4] or a continuous estrogen/intermittent progestin regimen (3 days on, 3 days off) [3]. In addition, other formulations are undergoing clinical trials or awaiting approval in the USA [3]. The

CI = confidence interval; EPRT = estrogen-progestin replacement therapy; ERT = estrogen replacement therapy; HRT = hormone replacement therapy; RR = relative risk.

most commonly used progestins in EPRT are the synthetic progestins, which can be divided into those structurally related to progesterone, which are most commonly used in the USA [5,6], and those that are structurally related to testosterone, which are frequently used in Europe and Scandinavia [7,8].

Progestins and breast cell proliferation

Hormones are hypothesized to increase cancer risk by increasing cell division, thereby increasing the risk for genetic errors of various kinds or fixing an initial mutagenic event. The vast majority of *in vitro* studies of normal breast cells in culture and breast cancer cell lines have shown that estrogens enhance breast cell proliferation, and that the addition of progestins reduces this effect [9]. In contrast, *in vivo* studies of the mitogenic effects of estrogens and progesterone on human breast epithelial cells in premenopausal women, which show a predominance of proliferative events during the luteal phase of the menstrual cycle when levels of both estrogens and progesterone are high, suggest that EPRT might have a more adverse effect on breast cancer risk than ERT [10]. Studies of epithelial cell proliferation in the normal postmenopausal breast in relationship to different regimens of HRT, however, have yielded discrepant results.

In a trial in which 40 postmenopausal women were randomly assigned to one of four treatment groups (daily topical application of a gel containing a placebo, estradiol, progesterone, or a combination of estradiol and progesterone during the 14 days preceding breast surgery) [11], progesterone reduced the estradiol-induced proliferation of breast epithelial cells. It is notable, however, that the drug regimens used in that study are not those typically used for HRT.

An observational study was conducted in which samples of breast tissue containing normal epithelium from 185 postmenopausal patients undergoing surgery for benign or malignant disease were stained for progesterone receptor and Ki67 expression [12]. There was no association between either estrogen or estrogen combined cyclically with progestin and breast epithelial cell proliferation. For patients on EPRT, information was not available regarding the cycle of treatment at the time of surgery.

In a second observational study of 86 postmenopausal women, combined/continuous EPRT was associated with greater epithelial cell proliferation and breast epithelial cell density than ERT or no HRT [13]. Moreover, the cell proliferation associated with EPRT was localized to the terminal duct lobular unit, where most breast cancers develop.

It is notable that estrogen plus progesterone has induced more pronounced proliferative responses than estrogens alone in the normal postmenopausal mammary gland in some murine models and macaques [9,14].

Hormone replacement therapy and breast cancer risk

Individual observational epidemiologic studies of HRT and breast cancer risk have yielded conflicting results. For instance, in a large prospective study [15], breast cancer risk was significantly increased among women who were currently using estrogen alone (relative risk [RR] 1.2, 95% confidence interval [CI] 1.1–1.5) or estrogen plus progestin (RR 1.4, 95% CI 1.2–1.7) as compared with postmenopausal women who had never used hormones. In a large case–control study [16], on the other hand, there was no increase in breast cancer risk associated with use of either estrogen alone or estrogen plus progestin. Such discrepancies may reflect the relatively small number of users of different types of hormone regimens even in the largest studies, as well as the relatively low levels of risk involved.

In a collaborative reanalysis of 90% of the world's epidemiologic data on HRT and breast cancer risk, which included data from 51 epidemiologic studies, increases in risk associated with HRT (without regard to type of hormone or regimen) were limited to current or recent users (those who stopped use 1–4 years previously) [17]. Among those women, the RR for each year of use was 1.023 (95% CI 1.011–1.036); the RR was 1.35 (95% CI 1.21–1.49) among women who used HRT for 5 or more years relative to never users. The increase in risk was greater among women with lower than among those with higher weight and body mass index, and cancers diagnosed in HRT users were less advanced clinically than those diagnosed in never users. Among the 39% of hormone users for whom information on type of preparation was available, 80% had primarily used preparations containing estrogen alone and 12% had used preparations containing both estrogen and progestin. The RR associated with 5 or more years of recent use of estrogen alone relative to never users was 1.34 (standard error 0.09), whereas the corresponding RR associated with use of estrogen and progestin or progestin alone was 1.53 (standard error 0.33). Analyses were not done according to type or regimen of progestin.

A number of subsequent observational epidemiologic studies have reported on breast cancer risk associated with EPRT as compared with ERT. Two of those studies [8,18] found similar increases in breast cancer risk associated with EPRT and ERT, although in the latter of the studies duration of EPRT use was shorter than duration of ERT use. Other studies [5–7,19–22] have suggested greater increases in risk with EPRT than with ERT. Among participants in the Nurses' Health Study [19], women with natural menopause who used ERT had a 7.7% (95% CI 5.0–10.5) increase in risk per year of use, whereas those who used estrogen plus progestin had a 13% (95% CI 7.2–19.1) increase in risk per year of use. The *P* value associated with the comparison of the rate of increase

with estrogen plus progestin versus estrogen alone was 0.06. In the Breast Cancer Detection Demonstration Project Follow-up Study [5], estrogen only and estrogen-progestin only were associated with 1% (95% CI 0.2–3) and 8.0% (95% CI 2–16) increases in risk per year of use, respectively, among users during the previous four years. The *P* value associated with the test of homogeneity of these estimates was 0.02. In a large case-control study [6], ERT was associated with a 6% (95% CI 0.97–15) increase in the RR for breast cancer for each 5 years of use, whereas EPRT was associated with a 24% (95% CI 7–45) increase. In another large case-control study [20], ERT and EPRT were associated with 2% (95% CI 1–3) and 4% (95% CI 1–8) increases in the RR per year of use, respectively. Progestin only use has been associated with a statistically significant increase in risk in two studies [8,20]. Several studies have suggested that EPRT is associated with greater increases in risk in leaner than heavier women [5,8], which is consistent with the collaborative reanalysis of the world's data [17]. Other studies have reported similar increases in risk in lean and heavy women [20,23]. Increases in risk have been noted for both testosterone-derived progestins [7,8] and progesterone-derived progestins [5–6,18–20,22].

Several observational epidemiologic studies have examined the association between EPRT and selected tumor characteristics. One noted an increase in risk only for lobular carcinomas [22], whereas several others reported greater increases in risk for lobular than ductal tumors [18,20]. Other studies, however, reported increases in risk with the vast majority of breast cancers with a ductal histology [5] or for both ductal and lobular carcinomas [23]. In two studies that examined risk according to the hormone receptor status of the tumors [18,23], increases in risk were evident for hormone receptor positive tumors but not for receptor negative tumors. One study [6] found similar increases in risk across all stages of disease with EPRT, whereas increases in risk associated with ERT were limited to *in situ* disease. Another study [20] found similar increases in risk for both localized and more advanced breast cancer.

Several observational epidemiologic studies have assessed breast cancer risk according to type of EPRT regimen. One study [8] reported greater increases in risk for the combined/continuous regimen than for the cyclic regimen (19%/year versus 3%/year). Other studies [18,20] reported similar increases in risk associated with the cyclic and combined/continuous regimens. Yet another study [6] found greater increases in risk with the cyclic than with the combined/continuous regimen (odds ratios per 5 years of use were 1.38 [95% CI 1.13–1.68] and 1.09 [95% CI 0.88–1.35], respectively), but this difference was not statistically significant. In a study conducted in Finland [4], where long cycle EPRT (adding a

progestin period every second or third month) has been used since 1990, both the long cycle and monthly cycle EPRT regimens were associated with statistically significant 30% increases in breast cancer risk. Risk was not evaluated as a function of dose or duration of use.

Results from two randomized controlled trials showed an increase in breast cancer risk associated with the combined/continuous estrogen-progestin regimen (0.625 mg conjugated equine estrogen plus 2.5 mg medroxyprogesterone acetate) [24,25]. The estrogen-progestin component of the Women's Health Initiative was stopped early, in part because of an increased risk of breast cancer in those taking estrogen plus progestin as compared with placebo (hazard ratio 1.26, 95% CI 1.00–1.59) [24]. A total of eight additional invasive breast cancers per 10,000 person-years were attributed to the estrogen-progestin regimen. Those findings are consistent with the relative hazard of 1.27 (95% CI 0.84–1.94) found after 6.8 years of follow-up in the Heart and Estrogen/Progestin Replacement Study Follow-up [25], a randomized trial in postmenopausal women with coronary disease.

Although HRT is associated with an increased incidence of breast cancer, it has been associated with lower mortality from breast cancer in most studies that examined breast cancer death among healthy hormone users as compared with nonusers, possibly reflecting more favorable tumor characteristics associated with HRT use [26]. Published data are insufficient to assess associations between the estrogen-progestin regimen, specifically, and breast cancer mortality.

Hormone replacement therapy and mammographic densities

Extensive areas of mammographic density, representing stromal or epithelial tissue, have been associated with substantially increased breast cancer risk. Although changes in mammographic density have not been examined in relationship to changes in breast cancer risk, changes in densities resulting from a variety of interventions suggest that mammographic densities may be a short-term marker of risk [27].

Most studies that examined changes in mammographic density according to type of HRT regimen have found that a greater percentage of women on EPRT than on ERT experienced increases in density [28–32]. In one of those studies [28], a double-blind randomized placebo-controlled trial, the percentages of women who experienced increases in density were similar among those taking cyclic and combined/continuous EPRT: 23.5% in those on the cyclic regimen with 10 mg medroxyprogesterone acetate; 19.4% in those on the combined/continuous estrogen-progestin with 2.5 mg medroxyprogesterone acetate; 16.4% in those on the cyclic regimen with

micronized progesterone; 3.5% in those taking estrogens alone; and 0% in the placebo group. In a Swedish study of changes in mammographic density assessed at two screening examinations [29], a greater percentage of women on combined/continuous EPRT than on cyclic EPRT experienced increases in mammographic density (28% and 10%, respectively), although both groups experienced greater increases in density than those on ERT (5%). Other studies [30–32] reported that greater percentages of women on combined/continuous, but not cyclic, EPRT exhibited increases in mammographic density as compared with women on ERT. For instance, in a study of 175 women participating in a population-based screening program [32], increases in mammographic density were observed in 52% of women receiving combined/continuous EPRT, in 13% of those receiving the cyclic regimen, and in 18% of those receiving ERT.

Conclusion

Taken together, these data suggest that the addition of progestins to ERT does not counteract the adverse effects of estrogens on the breast, as it does in the endometrium. In fact, the data suggest that EPRT may have a more adverse effect on risk for breast cancer than does ERT. Many issues remain unresolved, however. These include the effects of different regimens and doses of EPRT, long duration use, and whether the effects vary according to tumor characteristics, such as histology, extent of disease, and hormone receptor status. Data from several large studies that are currently underway may provide answers to some of these questions [33].

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