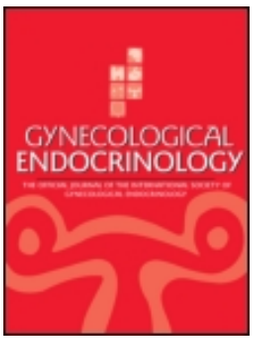

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Progestins and cancer

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Key words: ENDOMETRIUM, BREAST CANCER, PROGESTINS, HRT

ABSTRACT

The role of progestins in breast tissue is less well defined than in the endometrium. Although in vitro *studies have shown that progestins induce a similar decrease in both estrogen and progesterone receptors and an increase in 17β-estradiol dehydrogenase in the breast as in the endometrium, epidemiologic studies have suggested that progestins prevent endometrial cancer, but do not reverse the estrogen-related increase in breast cancer risk in long-term hormone-replacement therapy (HRT). Other studies have also suggested a protective effect for progestins on breast tissue.*

The dual effect of progesterone and progestins on the cell cycle has been demonstrated, suggesting that according to the duration of administration, the same steroid can induce cells to enter the multiplication phase or to enter a resting state.

Progestins exert different effects according to the steroid from which they are derived, e.g. pregnanes derived from progesterone, estranes or gonanes derived from testosterone. Some estrane derivatives are able to stimulate breast cell multiplication in vitro *through an estrogen receptor-mediated pathway. Most pregnanes do not exert such an effect. Also, some pregnane derivatives stimulate apoptosis, leading to cell death. However, it is well established that high doses of progestins have been successfully used in the treatment of advanced breast cancer as second-line endocrine therapy.*

Finally, striking differences have been observed in progestin use in Europe and in the USA. In France, where the rate of progestin use per head is higher than in the USA, the rate of breast cancer has not increased as sharply as observed in North America.

Although cancer genesis is multifactorial, it may be concluded that progestins do protect endometrial tissue against the proliferative action of estrogen and if they do not protect breast tissue, at least they do not stimulate its proliferation. Also, they are useful agents as a second-line therapy for breast cancer, when used at high doses.

PROGESTINS AND THE ENDOMETRIUM

It is well known that progesterone and progestins regulate the action of estradiol on endometrial cells and prevent endometrial hyperplasia. After a priming effect of estrogens, all progestins have been shown to mimic the action of progesterone and inhibit cell proliferation, decrease estrogen and progesterone receptors, and stimulate the effect of 17β-estradiol dehydrogenase which converts estradiol into estrone, a less active metabolite.

In hormone-replacement therapy (HRT), it is now well accepted that estrogens should be antagonized by co-administration of a progestin at doses and for a duration inhibiting endometrial proliferation. Epidemiological studies have now clearly shown that combined HRT decreases not only the risk of endometrial hyperplasia but also the risk of endometrial cancer[1].

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Epidemiological data on the relationship between progestins and the risk of endometrial cancer

The effects of HRT with estrogen alone on the risk for endometrial cancer are now reasonably well defined. Risk increases with increasing duration of use, reaching a relative risk of about 10 after ten or more years of use; the elevation in risk declines after cessation of use, but the risk is still significantly raised five or more years after last use[1].

Key[2] reviewed the results of six epidemiological studies analyzing the relationship of combined HRT with an estrogen and a progestin, and the risk of endometrial cancer. In all studies, use of estrogen with a progestin was associated with a lower relative risk than use of estrogen alone, but the risk was still higher than in untreated women. Both studies which presented results according to the duration of progestin use each month found that the relative risk was lower in women using progestins for ten or more days per month than in women using progestins for less than ten days per month.

For endometrial hyperplasia, evidence that 12–14 days of progestins are required to decrease the risk below 1% is undisputed[3,4]. Most studies involving sufficient numbers of patients and using various steroids showed that unopposed estrogens induce hyperplasia in 20–42% of cases, while the addition of a progestin would decrease the incidence of hyperplasia to about 1%.

PROGESTINS AND THE BREAST

Epidemiologic data and controversies about the role of progestins on breast cancer risk

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death among women in most developed countries. The annual incidence of female breast cancer increased by approximately 52% during the second half of the twentieth century, from 1950 to 1990, especially in the USA. However, the lifetime risk of dying from breast cancer has not changed and mortality curves have plateaued over the same period. It is recognized that the increased use of mammographic screening, helping to detect breast cancer earlier, could account for the apparent sharp increase in breast cancer cases in the late 1980s.

The relationship between female sex hormones and breast cancer incidence has been evaluated in a considerable number of epidemiological studies. Each time a new publication appears in the literature, controversy arises again. These discrepancies may be explained in both cases by the variety of study designs, populations studied, progestins used, and also the interpretation of observed results.

Estrogen-replacement therapy (ERT), when used for more than 10–15 years, may increase the risk of breast cancer by 30% according to a meta-analysis of epidemiological studies[5]. However, a causal relationship cannot be established and only large long-term randomized, placebo-controlled prospective trials can be definitely conclusive. Results from the Women's Health Initiative study, designed on such a basis, may afford an answer. However, this is unlikely to be until well into the next century.

Although many epidemiological studies have been conducted to assess the relationship between ERT and risk of breast cancer, only a few of them address specifically the role of progestins. The recent coprescription of progestins with estrogens for menopausal therapy, especially in the USA where most of the large epidemiological surveys were conducted, is of relevance here. Also, it must be remembered that progestins prescribed for HRT differ from country to country and their effects also differ according to the category to which they belong. In the USA, most prescriptions for progestins relate to medroxyprogesterone acetate, while in Europe derivatives of progesterone are preferred for HRT such as micronized progesterone, dydrogesterone, chlormadinone acetate derived from 17-hydroxyprogesterone, or nomegestrol acetate derived from 19-norprogesterone[6].

Therefore, several compounds are used in Europe and the population of HRT users would not be as homogeneous as it appears in the USA. In addition, estradiol-17β and estradiol valerate are more often prescribed in Europe than conjugated estrogens, which have been prescribed in the USA for some decades.

The controversy started with a publication evaluating risk of breast cancer in young women using oral contraceptives[7]. This study generated a huge number of other studies, both epidemiologic and experimental, examining the specific role of progestins on the mammary gland. Among the

epidemiological studies specifically examining the role of progestins and breast cancer, the first one published in the USA by Gambrell *et al.*[8] pointed out that progestins could reduce the risk of breast cancer. However this study was criticized as no adjustment was made for the other known risk factors for breast cancer.

The longest randomized and placebo-controlled experimental study was published by Nachtigall *et al.*[9] and here again it showed a lower number of cases of breast cancer in combined HRT users than in the placebo group. This study followed 84 matched pairs of women for more than 22 years. However, the number of patients involved was too small to draw definite conclusions.

In Scandinavia, two major studies appeared in the late 1980s. The study from Bergkvist *et al.*[10] raised controversy and public alarm since the results indicated that long-term users (for 9 years or more) of estrogens would have a relative risk (RR) of 1.7 as compared to non-users. Those who used the combined therapy would have a RR (interpreted in some media circles) as a fourfold increase in risk. Unfortunately, although it was stressed that a RR of 4.4 was not statistically significant, as the confidence interval (CI) was large (0.9–22.4, including the value 1), the popular press spread an alarming message.

Later, with further follow-up, the same Swedish group indicated that combined HRT users had a RR of 1.6 (statistically significant) but which was not much different from the risk observed with estrogen-only users[11]. Ewertz[12] did not find any statistically significant difference between estrogens taken alone or in combined HRT. The only category where a significant risk was observed in her study was the group of women receiving androgens combined with high doses of estrogens in implants.

Three recent studies show contradictory results. In the Nurses' Health Study[13] concerning 70 000 women followed for up to 16 years, the relative risk of breast cancer was significantly increased among women who were current users of estrogens (RR 1.32; CI 1.14–1.54) or used combined estrogens and progestins (RR 1.41; CI 1.15–1.74). No statistically significant difference was observed between the two groups using either estrogens alone or combined HRT. The main finding was of an earlier appearance of risk, after 5 years of use of HRT. In two population-based control studies published shortly after the Nurses' Health Study, no increase in risk in any group of HRT long-term users was observed[14,15].

These conflicting data have resulted in a lively debate as to whether progestins should be added to estrogens in HRT and especially whether hysterectomized women need progestins.

Striking differences have been observed in progestin use in Europe and in the USA. In France, where the rate of progestin use per head is higher than in the USA, breast cancer rates have not increased as sharply as observed in North America[6].

In a large French cohort study of premenopausal women with benign breast disease receiving high doses of progestins, Plu-Bureau *et al.*[16] showed a decreased risk in breast cancer in a group of women receiving 19-nortestosterone derivatives for at least 15 days per cycle. The other progestins did not decrease the risk significantly.

A consensus paper of the European Progestin Club[17] addressed the various arguments between countries and suggested new prospective studies with the newer types of progestins available in Europe. However, they concluded that progestins with a pharmacological profile comparable to that of natural progesterone would be preferable.

Experimental data in animals

Experimentally, there is much indirect evidence to suggest synergy and antagonism between estradiol and progesterone on mammary tissue. Which ovarian steroids stimulate normal human mammary epithelial cell proliferation is an important issue that can be addressed using the athymic nude mice model. McManus and Welsch[18] and, more recently, Laidlaw *et al.*[19] have analyzed the effects of estradiol and progesterone on normal human breast tissue implanted into athymic nude mice. They showed that estradiol stimulates human breast epithelial cell proliferation at physiologically relevant concentrations and progesterone does not affect proliferation either alone or after estradiol priming. They also demonstrated a dose–response relationship between proliferation and serum estradiol concentrations. Longman and Buehring[20] showed that progesterone or progestins when added alone did not stimulate cell growth in explants of normal mammary tissue. When

added to ethinylestradiol, progestins decreased cell growth[20].

Hormone-dependent mammary tumors can be induced in animal models by chemical carcinogens or irradiation. Animal studies have shown that induction of breast cancer by a chemical carcinogen is prevented when the mammary gland has undergone complete cell differentiation by a first full-term pregnancy before exposure to the carcinogen[21]. Experimentally, it was found that the dimethylbenzanthracene (DMBA)-induced tumor incidence was significantly decreased in animals previously treated with estradiol, progesterone or estradiol + progesterone, in that order of efficacy. It was also shown that combined administration of estradiol + progesterone induced tissue differentiation similar to that observed after a first full-term pregnancy, i.e. rapid and complete differentiation of all terminal end buds into alveolar buds, and then into lobules. The protective effect of progesterone against various carcinogens seems to be dependent upon the time of administration relative to the carcinogen and generally relates to stimulation by progesterone of terminal duct differentiation, thus protecting lobulo-alveolar glands from carcinogens[21].

Studies in primates

In *in vivo* studies performed in surgically postmenopausal female monkeys, it was suggested by Cline *et al.*[22] that medroxyprogesterone acetate administered orally, at doses equivalent to those used in women for HRT, would increase tissue proliferation. These authors indeed showed from morphometric studies that mammary gland thickness was increased under therapy, and also that the percentage of the mammary gland occupied by glandular tissue was increased more markedly in the group of animals receiving HRT. This finding was interpreted as a marker for a mitogenic role of the progestin. However, there was no statistical difference in the proliferative index measured in animals receiving estrogens alone or in combination with medroxyprogesterone acetate. In addition, as estrogen and progesterone act synergistically to promote tissue growth and acini formation, the increase in glandular volume may also account for the findings of Cline *et al.*[22].

Biochemical data

In experiments conducted on human breast adenoma cells, it was shown that progesterone regulates estradiol and progesterone receptors in epithelial cells[23]. It was also demonstrated that progesterone stimulates the enzyme 17β-dehydrogenase activity, which converts estradiol into estrone, a less active metabolite. The hormone dependence of those adenomas was confirmed in tumors with high epithelial content.

Using human breast cancer cell lines in culture, Catherino *et al.*[24] described the estrogenic activity of some 19-nortestosterone derivatives such as norgestrel and gestodene, which have been shown to stimulate breast cancer cell growth through an estrogen-receptor mechanism. Later, Catherino and Jordan[25] tested 19-norpregnane derivatives using the same model and showed that they inhibited cell growth and did not exhibit any estrogenic effect. Also, some pregnane derivatives were shown to stimulate the apoptotic process leading to cell death[26].

Progestins and the cell cycle

Musgrove *et al.*[27] have performed elegant *in vitro* studies demonstrating that breast cells in the last phase of cell cycle activity are initially driven to the S phase of DNA synthesis by progestins. This effect is transient and further application of progestins suppresses the cyclins, thus halting breast cell division in early G1 phase. These experiments underline a dual effect of progestins according to the duration of their administration, and may reconcile both hypotheses for the role of progestins: stimulator or suppressor of breast cell mitosis.

Human studies

In women, the effects of sex steroids on breast tissue are less well known than their effects on the endometrium, essentially because normal breast tissue is not easy to sample in humans. *In vivo*

studies have been designed to detect changes in breast structure and function during the menstrual cycle, and identify those which may predispose to malignant changes. The uptake of tritiated thymidine has been used for calculating a thymidine labeling index as a measure of the proliferative activity of normal breast tissue at different times of the cycle in women undergoing breast surgery[28].

In some studies, the mitotic activity of breast epithelial cells was reported to be higher during the follicular than during the luteal phase of a normal cycle, while other authors have shown a peak mitotic activity of epithelial cells and DNA synthesis in the late luteal phase[28]. As the midluteal phase of the cycle immediately follows the peak secretion of both progesterone and estrogen, it was inferred that progesterone is involved in the promotion of breast epithelial cell mitosis, in contrast to the well-documented antiproliferative effect of progesterone on the endometrium. However, this hypothesis remains controversial, as the exact timing of the biopsy within the menstrual cycle has not always been well defined. In addition, other authors argue that breast cells can react to the cumulative effect of estrogens and progesterone secreted over several cycles rather than to the change in hormonal secretion in the 24-hour period preceding the biopsy[6,29].

Interesting results on the interaction and effects of estradiol and progesterone on breast cell multiplication have recently been obtained *in vivo*[30]. Women undergoing surgery for benign breast disease were randomly treated with either estradiol or progesterone or placebo, applied locally to the breast between day 11 and 13 of the follicular phase of the menstrual cycle prior to surgery. The number of mitoses in the epithelial cells of the normal part of the breast was counted. After estradiol treatment, both estradiol concentrations in breast tissue and the number of mitoses were high. After progesterone administration, progesterone concentration in breast tissue was high, but the number of mitoses was low. In the placebo group, the number of mitoses was low. The authors concluded that *in vivo* high intratissue concentrations of progesterone were able to decrease the mitotic activity of the normal lobular epithelial cells.

Similar tests were conducted in postmenopausal women prior to breast surgery. Exposure to progesterone for 14 days reduced the estradiol-induced proliferation of normal breast epithelial cells *in vivo*[31].

PROGESTINS IN THE TREATMENT OF BREAST CANCER

Progestins have been used for many years as one element in the endocrine therapy of breast cancer[32]. The main therapeutic strategy is reduction of estrogen available to breast cells. The physiologic antiestrogenic effect of progesterone and its derivatives has been proposed and widely used.

The two main progestins used in clinical practice for treatment of advanced breast cancer are medroxyprogesterone acetate and megestrol acetate. When used at high doses, these compounds exert an antigonadotropic effect which has been observed both in pre- and postmenopausal women. A significant decrease in the sex hormone binding globulin (SHBG) has been observed with progestins, likely to be related to the decrease in estrogen level. Therefore, less estradiol is available for cellular uptake.

Another important effect of progestins is related to their ability to decrease the amount of estrone sulfate in breast tissue. Pasqualini *et al.*[33] have demonstrated that several progestins can block the conversion of estrone sulfate to estradiol very significantly in hormone-dependent breast cells.

The other antiestrogenic effect of progestins is exerted through their ability to decrease levels of progesterone receptors although non-receptor pathways have been described.

Therefore, potent progestational molecules may in theory be used in the endocrine treatment of breast cancer in order to decrease estrogen availability to cells.

In the strategy for the endocrine treatment of advanced breast cancer progestins are used only as second-line agents. The results of progestin therapy used as first-line therapy could be more encouraging as 40.2% (complete response + partial response) were reported with medroxyprogesterone acetate and 29.8% with megestrol acetate. However, the availability of several new aromatase inhibitors, affording similar or better response rates with fewer side-effects, may lead to a decrease in

the use of progestins such as these two agents in the management of breast cancer.

Nowadays, other potent progestins are available and have been shown to decrease gonadotropin levels both in post- and premenopausal women, and also induce a decrease in the production of estradiol, estrone and estrone sulfate[32,33]. Other new progestins, which exhibit potent antiestrogenic activity without androgenic or glucocorticoid effects, are possible treatments in the future.

Nevertheless, second-line therapy is proposed in patients with advanced disease, and whose prognosis is already compromised. The rate of response observed in such patients cannot be expected to be much higher than the 25% described in previous studies with medroxyprogesterone acetate and megestrol acetate. The only major advantage would be use of lower doses of these new potent agents for better tolerability.

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