Hormone Res. *28:* 212–218 (1987)


0301–0163/87/0284–0212$2.75/0

# Antiestrogen Action of Progesterone in Breast Tissue

*P. Mauvais-Jarvis, F. Kuttenn, A. Gompel*

Department of Reproductive Endocrinology, Necker Faculty of Medicine, Paris, France

**Key Words.** Progesterone · Breast cancer · Antiestrogen · Mammary gland · Ovarian function · Estrogen receptor · Cell multiplication

**Abstract.** This review analyzes recent data from international literature concerning the antiestrogen action of progesterone and progestins at the level of mammary cells in culture from either breast cancer lines or normal breast obtained from reduction mammoplasties. Most data indicate that progesterone and progestins have a strong antiestrogen effect on breast cell appreciated by the decrease of estradiol receptor content, the decrease of cell multiplication and the stimulation of 17β-hydroxysteroid activity which may be considered as a marker of breast cell differentiation dependent of progesterone receptor.

## Introduction

Many controversies have arisen recently in the literature about progesterone action in the breast [1–4]. These controversies are related to the possibility that an unopposed estrogen effect due to a defect in progesterone secretion during the luteal phase might be a promoting factor in human breast cancer genesis [1,2]. The fact that this hypothesis remains controversial is due to the insufficient knowledge on the antiestrogenic activity of progesterone and progestins in human breast cells. Whereas the information has been well documented for the endometrium [5, 6], only few data are available regarding breast cells. These recent data, obtained either in animal or in human breast target cells in culture, were convenient and demonstrated that breast target cells respond to the synergistic and antagonistic action of estradiol and progesterone in the same way as the endometrium.

## Experimental Data in Animals

Experimentally there are many indirect data to suggest an antagonism between estradiol and progesterone at the level of breast tissue. Many investigators have shown that the mammary gland of different species responds differently to estrogens if the hormone is administered in physiological or supraphysiological doses or in combination with progesterone or alone [7–11].

High doses of estrogen administered for a prolonged time to castrated female rats induces proliferation and dilatation of the lobules in the glandular tissue with formation of cysts and overgrowth of the epithelium [8]. In addition, estrogen provokes an increase of circumcanalicular and intralobular connective tissue [7]. The successive sequence of mammary alterations following the administration of estradiol to female rats is the proliferation of tubular system secretion, the dilatation of ducts, formation of cysts, and fibrosis [8]. These changes observed with supraphysiologic doses of estrogen seem to be comparable to human fibrocystic disease [7, 8]. In contrast, when estradiol is administered in combination with progesterone, complete and proper development of the mammary gland is observed when the ratio between estrogen and progesterone is adequate [10, 11]. Cowie et al. [9] found that a combination of estrogen and progesterone in castrated goats resulted in uniform development and secretion when the dose of estrogen remained low (0.25 mg/day). An increase in the estrogen dose to 1.0 mg/day resulted in cysts and epithelial proliferation.

## Biochemical Data

Biochemically, the antiestrogen activity of progesterone is well documented. The mechanism by which progesterone and progestins exert their antiestrogenic action in women include a reduction of estrogen secretion in the systemic circulation, an inactivation of estradiol by its metabolism at the target tissues, lowering estrogen receptor levels in these tissues [12], and a direct effect on cell multiplication.

*Effects on Blood Estrogen Levels*

Progestins in pharmacological doses may lower the circulating levels of estrogens by suppressing gonadotropins and ovarian function [13]. In particular, androstane derivatives are strong antigonadotropic agents [14]. In a large investigation performed in women with benign breast disease, we were able to confirm that lynestrenol administered from day 10 to day 25 of the menstrual cycle not only suppressed the ovulation peak of LH but also resulted in plasma estradiol levels less than or equal to 50 pg/ml [15].

*Effects on Estrogen Receptor Levels*

Another mechanism by which progestins can be antiestrogenic is their ability to lower estrogen receptor levels in the endometrium [5, 12, 16]. How this effect is mediated has not been biochemically elucidated. It has been suggested that the decrease in intracellular concentrations of estradiol, brought about by the increase in 17β-hydroxysteroid dehydrogenase (see below), may be responsible for the decline in estradiol-binding sites. Experiments in rats have shown, however, that progestins affect estrogen receptor levels, even though they do not influence the activity of the enzyme [18]. Progestins do not alter estradiol affinity for binding to its receptor, and it is likely that their effect involves a reduction of estrogen receptor synthesis. In the hamster uterus, progestins decrease the levels of nuclear estrogen receptors through processes inhibited by actinomycin or cycloheximide [16].

In human noncancerous tissue there is no direct proof that progesterone antiestrogenic activity is mediated through its action on the receptor. Only indirect information is available. First, in fibroadenomas removed at different times of the menstrual cycle, as re-

ported previously [19], there was a continuous decrease in the level of both cytosolic and nuclear estradiol receptors (ERc and ERn) throughout the luteal phase. In addition, when patients with fibroadenomas were treated 30 days before surgery with progestins (10 mg lynestrenol daily + percutaneous progesterone) their fibroadenomas did not contain any trace of estradiol receptor [unpubl. data].

*Effects on Breast cell Multiplication*

The opposite action of estradiol and progesterone on cell multiplication has been extensively studied in the endometrium, in which estradiol has a strong proliferative effect, whereas progesterone inhibits this effect and is actually involved in cell differentiation [5, 18, 20]. The first in vitro data were obtained on breast cancer cells by Vignon et al. [21] who observed that estradiol stimulated the growth of T47D breast cancer cells in culture whereas the progestin promegestone (R 5020) inhibited the cell growth induced by estradiol. In a recent study, Horwitz and Freidenberg [22] emphasized the role of the progesterone receptor (PR) in the control of breast cancer cell growth by using the breast cancer cell line T47 Dco, which is lacking ER and is antiestrogen resistant. In this model, R 5020 clearly suppresses cell growth with a parallel translocation of PR. This group made the same observation on the T47 Dco cell line, with RU 486 [20, 23], which, like other antihormones, seems to have both agonist and antagonist action on PR.

To the best of our knowledge, the only report on the respective effects of estradiol and progesterone on normal breast cells division is that of McManus and Welsh [24]. The studies were carried out on normal human breast tissue transplanted in athymic castrated nude mice and confirmed that estradiol stimulates normal breast cell growth whereas progesterone has no action.

In our laboratory, normal human epithelial cell cultures are currently obtained from reductive mammoplasties. These cells have been shown to be hormone dependent [25]. They provide a useful model for the study of the action of estradiol and progestins on breast cell division. Cell growth was estimated by daily cell counts using a histometric method providing histometric growth index and DNA assay [25]. Estradiol stimulation of cell growth obtained on secondary cultures could not be observed when the cells were cultured in the usual medium. This may be due to the presence of small amounts of free or conjugated estrogens contained in the 5% human serum added to the medium. Indeed, under minimal conditions of supplementation (i.e., 1% serum instead of 5%) and with only low amounts of insulin and EGF, a significant stimulation of cell growth by estradiol was evidenced and was dose dependent for estradiol concentrations ranging from $10^{-10}$ to $10^{-8}$ *M*.

Under optimal culture conditions, R 5020 slowed down cell proliferation, and this inhibition was dose dependent. In addition, the inhibition occurred in the absence as well as in the presence of estradiol. This result confirms data obtained by other groups on breast cancer cell lines in culture [20–22, 26], especially T47 Dco in which, despite the absence of ER, physiologic concentrations of progestins directly inhibit cell proliferation. However, it is interesting to note that, when estradiol was added to R 5020, cell growth inhibition was less effective than with R 5020 alone. This suggests that the specific proliferative effect of estradiol is preeminent

over a presumed estrogen-priming effect on PR levels [27, 28].

Interestingly, the antiprogesterone RU 486, when added to the medium with or without estradiol, wa also capable of inhibiting normal breast cell growth, but to a lesser extent than R 5020 since 10 *M* RU 486 is required to achieve the same effect as that observed with $10^{-9}$ *M* R 5020 [25]. This antiproliferative effect of RU 486 has already been observed in breast cancer cell lines [20, 23, 26]. The mechanism by which RU 486 inhibits breast cell growth remains unclear since this compound was first described as a progesterone antagonist in clinical trials and, as such, proposed as a contragestive or chemical precocious abortifacient [29]. However, the dual properties of antihormones and especially antiestrogens are well known [27, 30] and it seems probable that, like tamoxifen, RU 486 will have biphasic (agonistic and antagonistic) properties, depending on the presence of a pure progestin such as R 5020 in the medium. Indeed, when the culture medium is supplemented with an equimolar concentration or R 5020 and RU 486, it can be noted that cell growth inhibition decreases.

## Estrogen Metabolism at Target Level: The Importance of 17β-Hydroxysteroid Dehydrogenase

The high affinity of estradiol for its receptor suggests that the receptor level is the main factor in determining the amount of available hormone bound under physiologic conditions. However, other considerations suggest that hormone-metabolizing enzymes in the cell may interfere with the binding of the hormone by the receptor [17]. The main enzyme involved in the antiestrogenic activity is the progesterone-dependent 17β-hydroxysteroid dehydrogenase ($E_2DH$) [6, 31, 32]. The activity of the NAD-dependent $E_2DH$ plays, indeed, an important antiestrogen role since it converts a potent estrogen, estradiol, into a less active one, namely estrone. Indeed, the estrogen receptor has a lower affinity for estrone and the complex dissociates more rapidly [17]. Previous studies have demonstrated that $E_2DH$ activity was present in human endometrium and other tissues [6, 33–36]. Extensive investigations were therefore carried out in our laboratory in order to study the activity of this enzyme in normal breast cells. Primary cultures of normal breast cells prepared from surgical specimens of reduction mammoplasty were used to study the activity of the enzyme $E_2DH$ [37]. Epithelial cells and fibroblasts have completely different morphologies and growth rates. Epithelial cells are small, round and slightly near-shaped, with a massive nucleus which is round and very refringent. Fibroblasts are characteristically spindle-shaped with a small nucleus and filaments extending in several directions.

The kinetics of estrone formation after incubation of epithelial cells with $^3$H-estradiol for periods of time ranging from 15 to 40 min showed that the reaction is linear for the first 30 min. In fibroblast culture, estrone formation was much slower and a 24-hour incubation was necessary to obtain the same amount of estrone as after 1 h in epithelial cells. When estradiol was added to the culture medium, it had no effect on $E_2DH$ activity on either cell. The affinity and capacity of $E_2DH$ were greater in epithelial cells than in fibroblasts. In the absence of progestin treatment, $E_2DH$ activity increased slowly over the course of cell culture

whether estradiol was added or not [25]. Moreover, the stimulation of $E_2DH$ activity by the progestins MPA and R 5020 was only observed in a medium containing estradiol [23, 25]. An estrogen-priming effect is therefore necessary to the action of progestin on $E_2DH$ stimulation. This contrasts with the action of the progestin R 5020 on cell proliferation which can be observed even in the absence of estrogen supplementation to the medium. In addition, as already observed in human breast cancer cell lines [20, 23, 26], RU 486 results in different effects depending on the biological marker considered. It has progesterone-agonist properties and a partial antagonist effect on cell growth. Moreover, this RU 486 effect does not require the presence of estradiol. In contrast, if $E_2DH$ activity is used as a biological marker, RU 486 displays only an agonistic property and, as is the case for R 5020, requires the presence of estradiol.

In addition to their fundamental importance, the present results may have several implications concerning the genesis of breast cancer. They are in agreement with the hypothesis of a protective effect of progesterone or progestins against the mitogenic activity of estrogens in breast target tissue [1, 2]. Inversely, the fact that the progestin R 5020 inhibits breast cell multiplication may be opposed to the recent speculations of Pike et al. [3, 4] on the role of progestins contained in oral contraceptive preparations as factors increasing breast cell division. Whereas progestins actually inhibit breast cell division, the efficiency with which physiologic concentrations of progesterone counteract the mitogenic effect of estrogens is not as well documented. Progesterone, indeed, is much less efficient than R 5020 in culture [21]. This is only due to the fact that progesterone is markedly metabolized in cell cultures [20]. However, like R 5020, this natural steroid suppresses the replenishment of PR in T47 Dco cells in culture [22]. In addition, progesterone, when topically applied over the breast of patients with fibroadenomas, significantly increases $E_2DH$ activity measured in vitro in the epithelial cells of the tumors [33].

Several conclusions can be drawn from this study: (1) estradiol and the progestin R 5020 have an opposite effect on proliferation of normal human breast epithelial cells in culture; estradiol stimulates whereas R 5020 inhibits cell multiplication; (2) the antiproliferative action of R 5020 is effective even in the absence of estradiol; (3) apart from its antiproliferative effect, R 5020 appears to favor cell differentiation, inasmuch as the stimulation of $E_2DH$ enzymatic activity by progesterone or progestins is considered an index of such a differentiation; (4) the antiprogestin RU 486 has a dual progestin agonist/antagonist action, depending on the physiological response considered, and perhaps also on the presence on the DNA progesterone acceptor of another compound, binding more strongly or more specifically the site of the chromatin involved in transcriptional progesterone activity.

## References

1 Korenman, S.G.: The endocrinology of breast cancer. Cancer *46:* 874–878 (1980).
2 Mauvais-Jarvis, P., Sitruk-Ware, R.; Kuttenn, F.: Luteal phase defect and breast cancer genesis. Breast Cancer Res. Treat. *2:* 139–150 (1982).
3 Pike, M.C.; Henderson, B.E.; Krailo, M.D.; Duke, A.; Roy, S.: Breast cancer in young women and use in oral contraception: possible modifying effect of formulation and age at use. Lancet, *ii:* 926–929 (1983).

4 Pike, M.C.: Breast cancer and oral contraceptives. Lancet *ii:* 1180–1181 (1985).
5 Bayard, F.; Damilano, S.; Robel, P.; Baulieu, E.E.: Cystoplasmic and nuclear estradiol and progesterone receptors in human endometrium. J. clin. Endocr. Metab. *46:* 635–648 (1978).
6 Tseng, L.; Gusberg, S.B.; Gurpide, E.: Estradiol receptor and 17β-dehydrogenase in normal and abnormal endometrium. Ann. N.Y. Acad. Sci. *286:* 190–198 (1977).
7 Asboe-Hansen, G.: Hormonal effects on connective tissue. Physiol. Rev. *38:* 446–462 (1958).
8 Bassler, R.: The morphology of hormone induced structural changes in female breast. Curr. Top. Pathol. *53:* 1–89 (1970).
9 Cowie, A.T.; Folley, S.J.; Malpress, F.H.; Richardson, K.C.: Studies on the hormonal induction of mammary growth and lactation in the goat. J. Endocr. *8:* 64–88 (1952).
10 Eisen, M.J.: The occurrence of benign and malignant mammary lesions in rats treated with crystalline estrogen. Cancer Res. *2:* 632–644 (1942).
11 Lyons, W.R.; McGinty, D.A.: Effects of estrone and progesterone on male rabbit mammary glands. I. Varying doses of progesterone. Proc. Soc. exp. Biol. Med. *48:* 83–89 (1941).
12 Leavitt, W.W.; Chen, T.J.; Do, Y.S.; Carlton, B.D.; Allen, T.C.: Biology of progesterone receptors; in O'Malley, Birnbaumer, Receptors and hormone action, vol. II, pp. 157–188 (Academic Press, New York 1978).
13 Mauvais-Jarvis, P.; Sitruk-Ware, R.; Kuttenn, F.; Sterkers, N.: Luteal phase insufficiency: a common pathophysiologic factor in development of benign and malignant breast diseases; in Bulbrook, Taylor, Commentaries on research in breast disease, pp. 25–59 (Liss, New-York 1979).
14 Mauvais-Jarvis, P.: Current hormonal therapy of benign breast disease; in Krieger, Bardin, Current therapy in endocrinology, pp. 428–432 (Decker, Philadelphia 1983).
15 Mauvais-Jarvis, P.; Kuttenn, F.; Gompel, A.: Estradiol-progesterone interaction in normal and pathological cells. Ann. N.Y. Acad. Sci. *464:* 152–166 (1986).
16 Clark, J.M.; Hsueh, A.J.W.; Peck, E.J.: Regulation of estrogen receptor replenishment by progesterone. Ann. N.Y. Acad. Sci. *286:* 161–169 (1977).
17 Gurpide, E.: Antiestrogenic actions of progesterone and progestins in women; in Bardin, Milgrom, Mauvais-Jarvis, Progesterone and progestins, pp. 149–161 (Raven Press, New York 1983).
18 Clark, J.H.; Markaverich, B.M.: The effects of progesterone and dexamethasone on estradiol binding and uterine growth; in Bardin, Milgrom, Mauvais-Jarvis, Progesterone and progestins, pp. 163–177 (Raven Press, New York 1983).
19 Kuttenn, F.; Fournier, S.; Durand, J.C.; Mauvais-Jarvis, R.: Estradiol and progesterone receptors in human breast fibroadenomas. J. clin. Endocr. Metab. *52:* 1225–1229 (1981).
20 Horwitz, K.B.; Wei, L.L.; Sedlacek, S.M.; d'Arville, C.N.: Progestin action and progesterone receptor structure in human breast cancer: a review. Recent Prog. Horm. Res. *41:* 249–316 (1985).
21 Vignon, F.; Bardon, S.; Chalbos, D.; Rochefort, H.: Antiestrogenic effect of R 5020, a synthetic progestin in human breast cancer cells in culture. J. clin. Endocr. Metab. *56:* 1124–1130, 1983.
22 Horwitz, K.B.; Freidenberg, G.R.: Growth inhibition and increase of insulin receptors in antiestrogen resistant T47 Dco human breast cancer cells by progestins: implication for endocrine therapies. Cancer Res. *45:* 167–173 (1985).
23 Horwitz, K.B.: The antiprogestin RU 38486: receptor mediated and antiprogestin action screened in estrogen insensitive T47 Dco human breast cancer cells. Endocrinology *116:* 2236–2245 (1985).
24 McManus, M.J.; Welsh, C.W.: The effects of estrogen, progesterone and human placental lactogen on DNA synthesis on human breast ductal epithelium maintained in athymic mice. Cancer *54:* 1920 (1984).
25 Gompel, A.; Malet, C.; Spritzer, P.; Lalardrie, J.P.; Kuttenn, F.; Mauvais-Jarvis, P.; Progestin effect on cell, multiplication and β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. J. clin. Endocr. Metab. *63:* 1174–1180 (1986).
26 Bardon, S.; Vignon, F.; Chalbos, D.; Rochefort, H.: RU 486, a progestin and glucocorticoid antagonist inhibits the growth of breast cancer cells via the progesterone receptor. J. clin. Endocr. Metab. *60:* 692–697 (1985).
27 Horwitz, K.B.; Koseki, Y.; McGuire, W.L.: Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. Endocrinology *103:* 1742–1747 (1978).

28 Horwitz, K.B.; McGuire, W.L.: Estrogen control of progesterone receptor in human breast cancer. Correlation with nuclear processing of estrogen receptor. J. biol. Chem. *253:* 2223–2228 (1978).
29 Philibert, D.; Deraedt, R.; Teutsch, G.; Tounemine, C.; Sakiz, E.: RU 486: a new lead for steroidal antihormones. 64th Annual Meeting of the Endocrine Society, San Francisco 1982, abstr. 688.
30 Mowszowicz, I.; Bieber, D.E.; Chung, K.W.; Bullock, L.P.; Bardin, W.L.: Synandrogenic effects of progestins: comparison with non-progestational antiandrogens. Endocrinology *95:* 1589–1594 (1974).
31 Tseng, L.; Gurpide, E.: Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. Endocrinology *94:* 419–423 (1974).
32 Tseng, L.; Gurpide, E.: Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology *97:* 825–833 (1975).
33 Fournier, S.; Kuttenn, F.; De Cicco, F.; Baudot, N.; Mallet, C.; Mauvais-Jarvis, P.: Estradiol 17β-hydroxysteroid dehydrogenase activity in human breast fibroadenomas. J. clin. Endocr. Metab. *55:* 428–433 (1982).
34 Pollow, K.; Lubbert, N.; Boquoi, E.; Kreuzer, G.; Jeske, R.; Pollow, B.: Studies on 17β-hydroxysteroid dehydrogenase in human endometrium and endometrial carcinoma. Acta endocr. Copenh. *79:* 134–141 (1975).
35 Kreitman, O.; Kreitman-Gimbal, B.; Bayard, F.; Hodgen, G.D.: 17β-Hydroxysteroid dehydrogenase in monkey endometrium: characterization of enzyme activity and effects of estradiol alone or in combination with progesterone. Steroids *34:* 693–697 (1979).
36 Kreitman, O.; Bayard, F.; Hodgen, G.D.: 17β-Hydroxysteroid dehydrogenase in monkey endometrium during the menstrual cycle and at the time of implantation. Steroids *36:* 2674–2682 (1980).
37 Prudhomme, J.F.; Malet, C.; Gompel, A.; Lalardrie, J.P.; Boue, A.; Mauvais-Jarvis, P.; Kuttenn, F.: 17β-Hydroxysteroid dehydrogenase ($E_2DH$) activity in human breast epithelial cell and fibroblast in cultures. Endocrinology *114:* 1483–1489 (1984).

Pierre Mauvais-Jarvis, MD
Service de Médecine de la Reproduction
Hôpital Necker
149, rue de Sèvres
F–75730 Paris Cedex 15 (France)