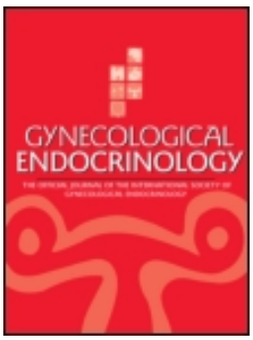

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Biological effects of progestins in breast cancer

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Key words: PROGESTINS, BREAST CANCER, SULFATASE; 17β-HYDROXYSTEROID DEHYDROGENASE, SULFOTRANSFERASE

ABSTRACT

Developments in the synthesis of different progestins have opened up new possibilities for the biological effects and therapeutic uses of these compounds. The actions of progestins are a function of their structure, affinity to the progesterone receptor or to other steroid receptors, the target tissue considered, the biological response, the experimental conditions, dose, and metabolic transformation. Data on the action of progestins in breast cancer patients are very limited. A positive response with the progestins medroxyprogesterone acetate and megestrol acetate has been obtained in postmenopausal patients with advanced breast cancer. However, extensive information on the effect of progestins was obtained in in vitro *studies using hormone-dependent and hormone-independent human mammary cancer cell lines. It was demonstrated that in hormone-dependent breast cancer cells, various progestins (nomegestrol acetate, medrogestone, promegestone) as well as tibolone, are potent sulfatase-inhibitory agents. Progestins may also be involved in the inhibition of the mRNA of this enzyme. In another series of studies, it was also demonstrated that various progestins are very active in inhibiting the 17β-hydroxysteroid dehydrogenase for the conversion of estrone to estradiol. More recently, it has been observed that promegestone or medrogestone stimulates the sulfotransferase for the formation of estrogen sulfates. Clinical trials of these enzymatic effects on the formation and transformation of estradiol in breast cancer patients could be the next step to investigate new therapeutic possibilities for this disease.*

INTRODUCTION

Recent statistical information indicates that in the USA, one woman in eight will be at risk of having breast cancer during her life; the values are one in 12 for countries of the European Community and one in 80 for Japan. About two-thirds of breast cancer cases are manifested during the postmenopausal period. The great majority (approximately 95%) are initially hormone dependent where the estrogen, estradiol, plays a predominant role in their development and evolution[1–3].

After a period that may last several years, the breast tumor becomes hormone independent by a very complex process, not yet fully elucidated. One explanation for the progression towards hormone independence may be the presence of estrogen-receptor (ER) mutants[4,5]. As the cancer cell evolves, mutations, deletions and truncations appear in the receptor gene[6–8], the ER becomes 'non-functional' and, despite estrogen binding, the cancer cells do not respond to the hormone; these data may explain why 35–40% of patients with ER-positive breast cancer fail to respond to antiestrogen therapy[9,10]. The remaining 5% of

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breast cancers are due to inheritance, for which the dominant susceptibility genes *BRCA-1* (on chromosome 17q21)[11,12] and *BRCA-2* (on chromosome 13q12-13)[13] have been identified.

Tissue concentrations of estrogens are very high in breast cancer patients, particularly during the postmenopausal period. It is well known that estrogen sulfate is the most important circulating estrogen during the menstrual cycle and in postmenopausal women[14,15]. Evaluation of unconjugated and sulfated estrogens in breast cancer tissue shows high values, particularly in postmenopausal patients[16]. Tumor : plasma concentration ratios show that the gradient for tumoral tissues to blood increases very significantly for estradiol and for estrogen sulfate when pre- and postmenopausal patients are compared (see Table 1).

There is abundant evidence that breast cancer tissues contain all the enzymes necessary for the formation of estradiol from circulating precursors, including sulfatase[17–22], aromatase[23,24], and 17β-hydroxysteroid dehydrogenase[25–28]. Breast cancer tissues also have the capacity to form estrogen sulfates from unconjugated estrogens. Estrogen sulfotransferase activity has been demonstrated in breast tumors[29,30] as well as in isolated human breast cancer cells[31]. Figure 1 gives a schematic representation of the activity of these enzymes in human breast cancer tissues. The available evidence leads to the 'intracrine concept', wherein hormones can be produced in the same organ in which the biological response takes place. This new concept of hormone action has also been developed in other hormone-dependent cancers, e.g. prostate cancer[32].

Two main pathways for estrogen formation in breast tumors have been identified: the aromatase pathway, which converts androgens into estrogens; and the sulfatase pathway, which converts estrogen sulfate into estrone. It has been demonstrated that tumoral sulfatase is 100–500 times higher than the aromatase activity[16].

ROLE OF PROGESTINS IN THE ENZYMATIC FORMATION AND TRANSFORMATION OF ESTRADIOL IN BREAST CANCER

Effect on estrone sulfatase

Intense estrone sulfatase activity has been demonstrated in breast cancer tissues as well as in isolated models, particularly in hormone-dependent cell lines[17–19,33–37].

Estrone sulfatase activity in mammary tumors is significantly higher in postmenopausal than in cycling patients and this activity is increased in tumor tissue compared to the surrounding area or in areas considered as normal[16]. In intact breast cancer cells in culture, a marked difference in sulfatase activity exists between hormone-dependent (MCF-7, T-47D) and hormone-independent (MDA-MB-231, MDA-MB-468) cells. The former possess high activity, whereas the latter have little effect on the hydrolysis of estrone sulfate[38,39]. When these hormone-independent

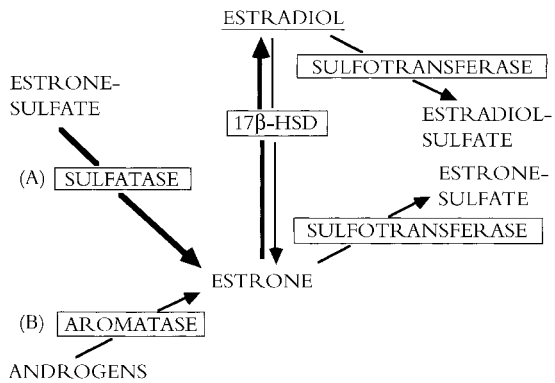

Figure 1 Enzymatic mechanisms involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (A) is quantitatively 100–500 times higher than that of the aromatas pathway (B). 17β-HSD, 17β-hydroxysteroid dehydrogenase

Table 1 Ratio concentration in tumor tissue and plasma of estrone, estradiol, and their sulfates in human breast cancer

Patients	*Estrone*	*Estradiol*	*Estrone sulfate*	*Estradiol sulfate*
Premenopausal	7	5	0.3	2
Postmenopausal	6	22	9.0	4

The ratio corresponds to values obtained with the tissue concentration of each estrogen (pmol/g) divided by the plasma concentration of the respective estrogen (pmol/ml). The data represent the average values obtained with

cells are homogenized, however, sulfatase activity is restored[22,40]. The data suggest that in the mechanism involved in the hydrolysis of the sulfate, 'stimulatory factors' necessary for the enzyme activity are present in the hormone-dependent, but could be absent in the hormone-independent, cells.

The effect of progestins (see Table 2 for their classification) on sulfatase activity in breast cancer has, to date, only been explored in *in vitro* studies. In breast tumors, progestins such as demegestone or chlormadinone acetate, at 10^{-5} mol/l, inhibit sulfatase activity by 25–50%[41,42]. In breast cancer cells (MCF-7, T-47D), promegestone (R-5020), nomegestrol acetate, medrogestone, tibolone, norethisterone, as well as danazol, at a range of concentrations between 5×10^{-8} and 5×10^{-5}, are potent antisulfatase agents which can decrease sulfatase activity by 40–90%[43–49]. As an example, Figure 2 shows the inhibitory effect of medrogestone on the sulfatase activity in MCF-7 and T-47D breast cancer cells. In homogenates of breast cancer cells (MCF-7, T-47D), R-5020 is a

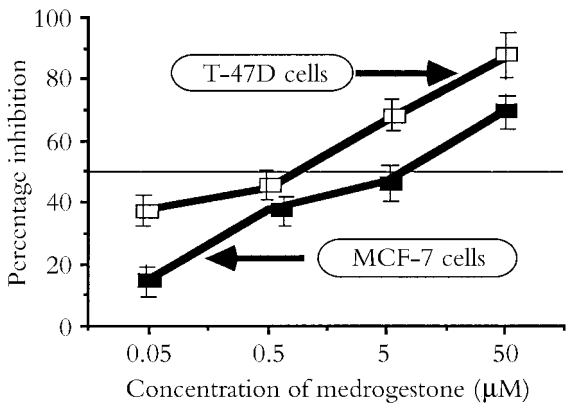

Figure 2 Dose–response effect of medrogestone on the conversion of estrone sulfate to estradiol in MCF-7 and T-47D human hormone-dependent breast cancer cells. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone sulfate ([^{3}H] estrone sulfate: 5 nM) alone (control) or in the presence of medrogestone (range: 0.05–50 μmol/l). Results (pmol estradiol formed/mg DNA) are expressed as a percentage of control values by calculating the ratio: [(control − test)/control] × 100. The data are the means ± SEM of duplicate determinations of three or four experiments. The IC_{50} values correspond to the concentration of medrogestone needed to obtain 50% inhibition vs control ([^{3}H] estrone sulfate alone): for T-47D cells, 0.85 μmol/l; for MCF-7 cells, 9.95 μmol/l

competitive inhibitor of sulfatase[50]. This progestin can also decrease the expression of sulfatase mRNA in both cells. A correlation with the reduction of the enzymatic activity was observed[51].

Effect of progestins on 17β-hydroxysteroid dehydrogenase activity

17β-Hydroxysteroid dehydrogenase catalyzes bidirectional reactions (oxidative activity: estradiol → estrone; and reductive activity: estrone → estradiol) and can be involved in both the inactivation or the synthesis of estradiol, respectively. It is now known that the enzyme consists of a number of isoenzyme forms (I to V) which express different activities, substrate specificities, regulations and localizations[52]. This aspect is very complex due to the fact that the orientation of the enzymatic activity (oxidative or reductive) is dependent on the nature and concentration of the co-factors [NAD(P) or NAD(P)H] produced by the cells[53,54]. In normal breast tissue in culture, it was observed that the oxidative enzymatic activity (estradiol → estrone) is the preferred direction and that this activity is more intense during the secretory phase of the menstrual cycle[55]. The isoform type I of the 17β-hydroxysteroid dehydrogenase family is specific to estrogens, possesses a preferential reductive activity and is essentially expressed in breast cancer tissues and estrogen-sensitive breast cancer cell lines[56,57]. However, types II and IV, with oxidative preferential activity, have also been detected at low levels in several cell lines (T-47D, BT-20, MDA-MB-231, MDA-MB-436)[58]. Studies on estrogen metabolism have demonstrated that the enzymatic reductive activity is very high in hormone-dependent breast cancer cells (MCF-7, T-47D) whereas in hormone-independent cells (MDA-MB-231, MDA-MB-468) the oxidative activity is preferred[28,59], suggesting that there is a change in 17β-hydroxysteroid dehydrogenase phenotype in neoplastic cells[60].

Various progestins have been tested in order to explore their effect on 17β-hydroxysteroid dehydrogenase in breast cancer cells. Interesting data have been obtained with nomegestrol acetate, medrogestone and tibolone. All these substances significantly decrease the conversion of estrone to estradiol in hormone-dependent breast cancer cells[43,45,61]. As an example, Figure 3 shows the inhibitory effect of medrogestone on the

conversion of estrone to estradiol in MCF-7 and T-47D cells. This effect is more intense with progesterone receptor (PR)-rich T-47D cells. Promegestone (R-5020) has no effect on this reductive activity but can increase the oxidative activity (estradiol → estrone) of 17β-hydroxysteroid dehydrogenase in T-47D cells[28,59]. The data suggest that there is a change in 17β-hyroxysteroid dehydrogenase phenotype in this kind of neoplastic cell[60].

Other authors have obtained an increase of both the reductive and the oxidative 17β-hydroxysteroid dehydrogenase activities with progesterone, medroxyprogesterone acetate, levonorgestrel and norethisterone in MCF-7 cells[61,62]. Medroxyprogesterone acetate also stimulates these dual activities in ZR-75–1 cells, whereas Org 2058 increases the oxidative direction in T-47D cells[63,64].

Effect of progestins on aromatase activity

The aromatase cytochrome P450 catalyzes aromatization of androgens to estrogens; biochemical and immunocytochemical studies have revealed the presence of this enzyme in adipose stromal cells of breast cancer tissues. Although levels of aromatase activity are relatively low in breast tissue, this local production of estrogens 'on site' can contribute to the pathogenesis of estrogen-dependent breast cancers. Aromatase inhibition by antiaromatase (e.g. by aminoglutethimide, 4-hydroxyandrostenedione, Letrozole® (Ciba Geigy Ltd, Basel, Switzerland), Arimidex® (Zeneca Ltd, Wilmington, DE, USA) is a well-defined second-line therapeutic treatment for breast cancer in postmenopausal women[65–68]. Studies of the control of aromatase by progestins are very limited. Perel *et al.*[69] observed that progestins can inhibit aromatase activity in cultured breast carcinoma cells.

Control by progestins of sulfotransferase activity

Steroid sulfotransferases, which convert estrogens into their sulfates are also present in breast cancer[19,22,70,71]. Sulfotransferase activity is important, firstly, because the high concentration of sulfoconjugates creates a reservoir of precursors for the biosynthesis of biologically active estradiol

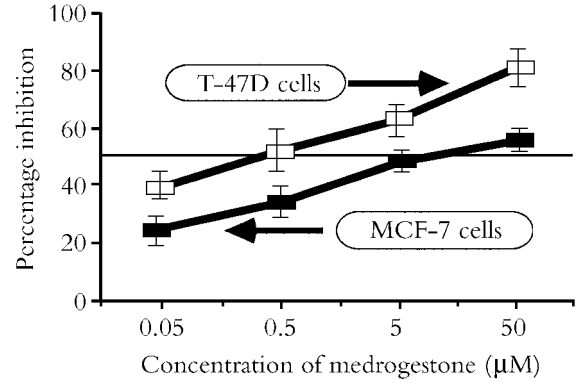

Figure 3 Inhibitory effects of medrogestone on the conversion of estrone (E_1) to estradiol (E_2) in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. Preconfluent cells were incubated for 24 h at 37°C with a physiological concentration of estrone ([^{3}H]-E_1: 5×10^{-9} mol/l) alone (control: non-treated cells) or in the presence of medrogesterone at a concentration range from 0.05 to 50 μM. The percentage of the inhibitory effect (in pmol E_2 formed/mg DNA) was obtained by calculating the ratio: [(control – test)/control] × 100. The data are the mean ± SEM of duplicate determinations of 3–4 independent experiments. The IC_{50} values correspond to the concentration of medrogestone needed to obtain 50% inhibition vs control ([^{3}H]-E_1S alone); for T-47D cells, 0.45 μmol/l; for MCF-7 cells, 17.36 μmol/l

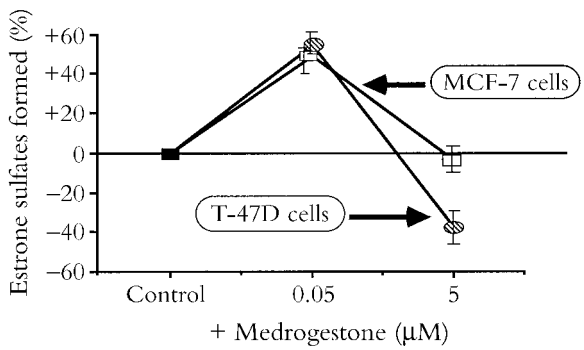

Figure 4 Dose–response effect of medrogestone on the conversion of estrone to estrogen sulfates in MCF-7 and T-47D human hormone-dependent breast cancer cells. Preconfluent cells were incubated for 24 h at 37°C with estrone ([^{3}H] estrone: 5 nM) alone (control) or in the presence of medrogestone (range: 0.05–50 μM). Results (pmol estrone sulfate formed/mg DNA) are expressed as a percentage of control values by calculating the ratio: [(control – test)/control] × 100. The data are the means ± SEM of duplicate determinations of three experiments

Table 2 Classification of progestins, their commercial name and pharmaceutical laboratories

Structure	*Progestin*	*Commercial name*	*Laboratory*
Pregnane derivatives			
Progesterone and derivatives	Progesterone	Utrogestan	Besins-Iscovesco
	Medrogestone	Colprone-5	Solvay (Wyeth France)
Retroprogesterones	Dydrogesterone	Duphaston 10	Solvay
17α-Hydroxyprogesterone derivatives	Medroxyprogesterone acetate	Depo-provera	Upjohn
	Medroxyprogesterone caproate	Pherlon	Schering
	Megestrol acetate	Megace	BMS
	Chlormadinone acetate	Luteran	Solymes
	Cyproterone acetate	Androcar	Schering
19-Nor-progesterone derivatives	Demegestone	Lutionex	Roussel
	Promegestone	Surgestone	Cassenne
	Trimegestone	*	HMR/Wyeth
17α-Hydroxy-nor-progesterone derivatives	Gestonerone caproate	Depostat	Schering
	Nomegestrol acetate	Lutenyl	Theramex
Androstane and estrane derivatives			
19-Nor-testosterone derivatives	Norethisterone	Norluten	SKF
	Norethisterone acetate	Primolut-Nor	Schering
	Norethisterone enanthate	Noristerat	Schering
	Lynestrenol	Exluton, Orgametril	Organon
	Ethynodiol diacetate	Lutometrodiol	Searle
	Norethynodrel	*	Searle
	Levonorgestrel	Microval	Wyeth-Byla
	Gestodene	*	Schering
	Desogestrel	Varnoline	Organon
	Dienogest	*	Jenapharm
	Norgestrienone	Ogyline	Roussel/HMR
	Gestrinone	Dimetriose	Roussel/HMR
	Norgestimate	*	Ortho

*These progestins are applicable only when associated with estrogens in combined progestative contraceptive pills and are not marketed on their own

through the action of endogenous sulfatase and, secondly, because sulfoconjugates are biologically inactive and do not bind to the estrogen receptor (ER).

Limited information is available on the effect of progestins on sulfotransferase activity in breast cancer. Very recent data have shown that the progestin promegestone (R-5020) and medrogestone at low concentrations (5×10^{-7}–5×10^{-8} mol/l) can increase the sulfotransferase activity in hormone-dependent breast cancer cells MCF-7 and T-47D, while higher concentrations (5×10^{-5} mol/l) decrease this activity. This dual effect is correlated with the mRNA expression of estrogen sulfotransferase (EST) which is modulated by promegestone in a similar manner[72]. Figure 4 shows the effect of medrogestone on sulfotransferase activity in MCF-7 and T-47D breast cancer cells.

CONCLUSION AND PERSPECTIVES

Data on the action of progestins in breast cancer patients are very limited. A positive response using the progestins medroxyprogesterone acetate and megestrol acetate has been obtained in postmenopausal patients with advanced breast cancer.

In breast cancer, one of the main therapeutic strategies consists of reducing the amount of estrogen available to breast-tissue cells. Very attractive data have been obtained concerning the action of various progestins (promegestone; nomegestrol acetate; medrogestone), as well as tibolone on the inhibition of enzymes involved in the formation of estradiol in breast cancer cells. This includes the inhibitory effects on sulfatases and 17β-hydroxysteroid dehydrogenase.

More recently, it has been found that some progestins (promegestone; medrogestone) can stimulate sulfotransferase activity. This is an important point in the physiopathology of breast cancer because it is well known that the estrogen sulfates are biologically inactive.

In addition to the use of antiaromatase agents to block estrogens, the utilization of various progestins in trials with breast cancer patients, showing an inhibitory effect on sulfatases and 17β-hydroxysteroid dehydrogenase (type I), and a stimulatory effect on sulfotransferases, will provide a new possibility in the treatment of this disease.

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