Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 93 (2005) 201–208
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

The role of progesterone metabolites in breast cancer: Potential for new diagnostics and therapeutics☆

J.P. Wiebe*, M.J. Lewis, V. Cialacu, K.J. Pawlak, G. Zhang

Department of Biology, Hormonal Regulatory Mechanisms Laboratory, University of Western Ontario, London, Ont., Canada N6A 5B7

Abstract

Proliferative changes in the normal breast are known to be controlled by female sex steroids. However, only a portion of all breast cancer patients respond to current estrogen based endocrine therapy, and with continued treatment nearly all will become unresponsive and experience relapse. Therefore, ultimately for the majority of breast carcinomas, explanations and treatments based on estrogen are inadequate. Recent observations indicate that 5α-pregnane and 4-pregnene progesterone metabolites may serve as regulators of estrogen-responsive as well as unresponsive human breast cancers. The conversion of progesterone to the 5α-pregnanes is increased while conversion to the 4-pregnenes is decreased in breast carcinoma tissue, as a result of changes in progesterone metabolizing 5α-reductase, 3α-hydroxysteroid oxidoreductase (3α-HSO) and 20α-HSO activities and gene expression. The 5α-pregnane, 5α-pregnane-3,20-dione (5αP) stimulates, whereas the 4-pregnene, 3α-hydroxy-4-pregnen-20-one (3αHP), inhibits cell proliferation and detachment, by modulation of cytoskeletal and adhesion plaque molecules via the MAP kinase pathway and involving separate and specific plasma membrane-based receptors. The promotion of breast cancer appears to be related to changes in in situ concentrations of cancer-inhibiting and cancer-promoting progesterone metabolites. New diagnostic and therapeutic possibilities for breast cancer are suggested.

Keywords: Progesterone metabolites; Breast cancer; Steroid receptors; Membrane steroid receptors; Gene expression; Human breast cell lines; MCF-7, MCF-10A, T47D, MDA-MB-231; 5α-Reductase; 3α-Hydroxysteroid oxidoreductase, 20α-hydroxysteroid dehydrogenase; 5α-Dihydroprogesterone; 3α-Dihydryprogesterone; MAP kinase; Actin Vinculin; Proliferation; Adhesion

1. Introduction

Breast cancer is one of the most widespread malignancies of women in Western society. In spite of extensive investigations, there is currently no adequate endocrine explanation for the majority of breast cancer cases. Although both female sex steroids, estradiol-17β (estradiol) and progesterone, are known to be involved in normal breast development as well as in the proliferative changes that occur during the menstrual cycle, pregnancy and lactation [1,2], current endocrine therapy is based almost exclusively on suppression of estradiol action. However, this estrogen-based therapy is effective in only a fraction of all breast cancer patients. Moreover, a large proportion of those patients with advanced neoplasia who respond initially, will eventually experience relapse and fail to respond to additional anti-estrogen therapy [3]. Thus, for the large number of breast cancers that are unresponsive to estrogen-based therapy, as well as for those showing relapse, there is currently no adequate hormonal explanation and hence no hormone-based treatment. The role of progesterone in breast cancer is not understood and although progesterone metabolism is known to occur in breast tissue, the potential role of progesterone metabolites has only recently begun to be explored [4].

The impetus for research on the metabolites of progesterone stemmed from the numerous conflicting reports about the effects of progesterone and other (synthetic) progestins on breast cancer cells. For example, some studies showed tumour regression with some doses of progestin treatment [5], while others reported increased epithelial proliferation [6]. Retrospective studies suggested that surgery performed

☆ Proceedings of the 16th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology, "Recent Advances in Steroid Biochemistry and Molecular Biology" (Seefeld, Tyrol, Austria, 5–8 June 2004).

* Corresponding author. Fax: +1 519 661 3935.
E-mail address: jwiebe@uwo.ca (J.P. Wiebe).

0960-0760/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.12.003

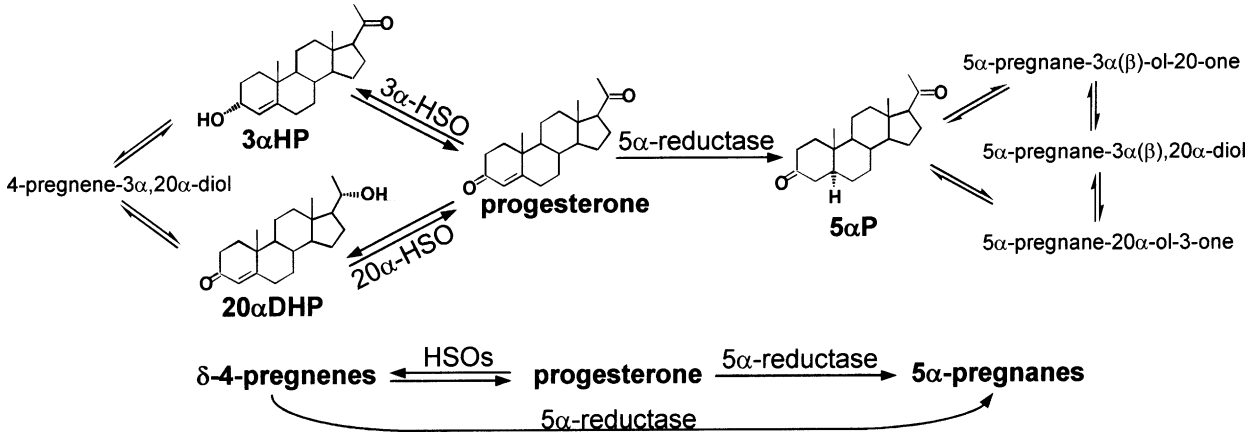

Fig. 1. Progesterone conversion to d-4-pregnenes and 5α-pregnanes by human breast tissue.

during the luteal phase of the menstrual cycle (when progesterone levels are higher) resulted in higher disease-free and overall survival rate than when surgeries were performed during the follicular phase [7]. In vivo, progestins either stimulate or decrease tumour growth [8] and in vitro either stimulate or inhibit cell proliferation [9], and cell cycle progression [10]. These reported contrary, and seemingly paradoxical, actions of progesterone in breast cancer cells, suggested to us the possibility that progesterone may be converted within breast tissue to two types of metabolites, those that stimulate and those that inhibit cell proliferation and tumorigenesis. If these could be shown to exist, they might also provide the basis of an endocrine explanation for all those breast cancer cases which are unresponsive to the anti-estrogen therapy. In our studies we identified the progesterone metabolites produced by breast tissue, the differences in progesterone metabolite production and in metabolizing enzyme activity and mRNA expression in normal and carcinoma tissue, the effects of the metabolites on cell proliferation and adhesion, cellular and molecular mechanisms of action and the unique receptors for the progesterone metabolites. The findings suggest potential roles of the progesterone metabolites in breast cancer.

2. Progesterone metabolism in normal and tumor breast tissue

Tumorous and non-tumorous tissues from the operated breasts of patients were used to determine their relative capacities for converting [^{14}C]progesterone. All the breast tissues in the study converted [^{14}C]progesterone into at least ten different metabolites [4], regardless of estrogen (ER) and progesterone (PR) receptor concentrations or a woman's age and ovarian state. The metabolites were rigorously identified by procedures that included TLC, HPLC, chemical derivatization, gas chromatography and mass spectrometry [4,11]. The results showed that breast tissues convert progesterone into two classes of metabolites (illustrated in Fig. 1): those which retain the double bond of progesterone in the carbon-4 position of ring A (delta-4-pregnenes; 4-pregnenes) and those that are 5α-reduced (5α-pregnanes). In the progesterone conversion pathway, the first 5α-reduced metabolite is 5α-pregnane-3,20-dione (5αP), catalyzed by 5α-reductase activity. The two 4-pregnenes resulting from direct progesterone conversion are 4-pregnen-3α-ol-20-one (3αHP) and 4-pregnen-20α-ol-3-one (20αDHP), catalyzed by the actions of 3α-hydroxysteroid oxidoreductase (3α-HSO) and 20α-HSO, respectively (Fig. 1). The conversion to 5αP is irreversible, whereas the conversions to 3αHP and 20αDHP are reversible reactions. Each of the 4-pregnenes can be further reversibly converted to 4-pregnene-3α,20α-diol. 5αP can be altered to 3- and 20-hydroxy pregnanes by the reversible actions of 3α-HSO, 3β-HSO, and 20α-HSO. Each 4-pregnene can also be irreversibly 5α-reduced to the respective 5α-pregnane by the action of 5α-reductase (Fig. 1). Thus the ratio of 5α-pregnanes:4-pregnenes can be altered by changes in activities of the progesterone metabolizing enzymes (PMEs), 5α-reductase, 3α-HSO and 20α-HSO.

3. Progesterone conversion to 5α-pregnanes is increased and conversion to 4-pregnenes is decreased in tumorous compared to normal (nontumorous) breast tissues

Although both normal and tumorous breast tissues convert progesterone to the two classes of metabolites, there are significant quantitative differences [4]. In normal (nontumorous) breast tissue, conversion of progesterone to 4-pregnenes greatly exceeds the conversion to 5α-pregnanes, whereas in tumorous tissue, production of 5α-pregnanes is higher than that of 4-pregnenes (Fig. 2a). The average ratio of 5α-pregnanes/4-pregnenes increases more than five-fold, from about 0.3 in non-tumorous to about 1.6 in tumorous breast tissue. The differences in amounts of 4-pregnenes and 5α-pregnanes are mainly due to changes in the amounts of the metabolites, 3αHP and 5αP, and the ratio of 5αP:3αHP is nearly 30-fold higher in tumorous than in nontumorous breast tissues (Fig. 2b).