Molecular and Cellular Endocrinology 301 (2009) 59–64

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Disturbed estrogen and progesterone action in ovarian endometriosis

Tina Šmuc[a,*], Neli Hevir[a], Martina Ribič-Pucelj[b], Bettina Husen[c], Hubert Thole[c], Tea Lanišnik Rižner[a]

[a] Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia
[b] Department of Obstetrics and Gynecology, University Medical Centre, Ljubljana, Slovenia
[c] Solvay Pharmaceuticals Research Laboratories, Hannover, Germany

ARTICLE INFO

Article history:
Received 1 July 2008
Received in revised form 22 July 2008
Accepted 22 July 2008

Keywords:
Endometriosis
17β-Hydroxysteroid dehydrogenases
AKR1C1
AKR1C3
Estrogen- and progesterone-metabolizing enzymes
Estrogen and progesterone receptors

ABSTRACT

Endometriosis is a very common disease in pre-menopausal women, where defective metabolism of steroid hormones plays an important role in its development and promotion. In the present study, we have examined the expression of 11 estrogen and progesterone metabolizing enzymes and their corresponding receptors in samples of ovarian endometriomas and control endometrium. Expression analysis revealed significant up-regulation of enzymes involved in estradiol formation (aromatase, sulfatase and all reductive 17β-hydroxysteroid dehydrogenases) and in progesterone inactivation (AKR1C1 and AKR1C3). Among the estrogen and progesterone receptors, ERα was down-regulated, ERβ was up-regulated, and there was no significant difference in expression of progesterone receptors A and B (PRAB). Our data indicate that several enzymes of estrogen and progesterone metabolism are aberrantly expressed in endometriosis, which can lead to increased local levels of mitogenic estradiol and decreased levels of protective progesterone. Changes in estrogen receptor expression suggest that estradiol may also act via non-estrogen receptor-mediated pathways, while expression of progesterone receptors still needs further investigation.

1. Introduction

Endometriosis is a complex disease that is defined as the presence of endometrial glands and stroma outside the uterine cavity and musculature (Olive and Schwartz, 1993). It is most commonly diagnosed in women of reproductive age (Berkley et al., 2005). There are three different types of endometriosis, namely ovarian endometriosis, peritoneal endometriosis and deep endometriotic nodules, which represent three different entities with different pathogenesis (Nap et al., 2004). Pathophysiological mechanisms have been extensively studied on animal models, but due to species-specific metabolism and reproductive physiology they still cannot replace studies on human samples (Grümmer, 2006).

In general, endometrial implants behave like normal endometrium in their response to hormones, where estradiol (E2) stimulates proliferation and progesterone (P) promotes differentiation (Olive and Schwartz, 1993). In pre-menopausal women the ovaries are the primary sources of E2, but E2 can also be produced in peripheral tissue from inactive precursors (Labrie et al., 2000). Locally, in endometriotic tissue, E2 can be synthesised in two ways: by the aromatase pathway, which includes conversion of ovarian or adrenal androstenedione to the weakly estrogenic estron (E1); this is further converted to active E2 (Bulun et al., 1999, 2000, 2001) by 17β-hydroxysteroid dehydrogenases (17β-HDSs) types 1, 7 and 12. The reverse reaction is catalyzed by the oxidative 17β-HSDs types 2, 4 and 8 (Luu-The, 2001; Penning, 2003; Midnich et al., 2004; Vikho et al., 2004; Luu-The et al., 2006).

The use of aromatase inhibitors for the treatment of endometriosis has been successful, as this is the rate-limiting step in local E2 production (Bulun et al., 2001; Patwardhan et al., 2008). The other pathway, the so-called sulfatase pathway, includes sulfatase and sulfotransferase. Sulfatase converts estrogen sulfate to E1, which should be activated by the reductive 17β-HSDs, while sulfotransferase inactivates E1 and E2, forming their sulfates. The production of estrogens from circulating levels of estrogen sulfate is another important alternative or complementary pathway to ovarian steroidogenesis.

P action can also be regulated at the pre-receptor level. Locally P can be converted to the less active 20α-hydroxyprogesterone (20α-OHP) and/or to 5α-reduced metabolites (Pollow et al., 1975). The major human reductive 20α-HSDs are aldo–keto reductases AKR1C1 and AKR1C3, with the latter also acting as a reductive 17β-

* Corresponding author. Tel.: +386 1 5437593; fax: +386 1 5437588.
E-mail address: Tina.Smuc@mf.uni-lj.si (T. Šmuc).

0303-7207/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2008.07.020

Table 1
Patients details: endometriosis group

Sample	Age	Diagnosis	Phase of menstrual cycle
13	33	*Endometriosis ovarii*	Proliferative
14	24	*E. ovarii*	Proliferative
15	33	*E. ovarii*	Late proliferative/early secretory
16	30	*E. ovarii*	Late proliferative/early secretory
18	44	*E. ovarii*	Proliferative
19	27	*E. ovarii*	Early secretory
20	30	*E. ovarii*	Late proliferative/early secretory
21	34	*E. ovarii*	Proliferative
22	32	*E. ovarii*	Secretory
23	27	*E. ovarii*	Secretory
24	36	*E. ovarii*	Proliferative
25	33	*E. ovarii*	Proliferative
26	28	*E. ovarii*	Late proliferative/early secretory
27	40	*E. ovarii*	Proliferative
28	29	*E. ovarii*	Secretory
29	39	*E. ovarii*	Late proliferative/early secretory
30	31	*E. ovarii*	[a]
31	28	*E. ovarii*	Secretory
32	37	*E. ovarii*	[a]
36	47	*E. ovarii*	Proliferative
37	28	*E. ovarii*	Proliferative
41	37	*E. ovarii*	Secretory
43	26	*E. ovarii*	[a]
45	25	*E. ovarii*	Proliferative

[a] Not determined.

HSD and potentially also involved in E2 synthesis (Penning et al., 2000).

The abundance and distribution of estrogen receptors (ERs) and progesterone receptors (PRs) will determine whether E2 or P will have a particular effect (Pearce and Jordan, 2004). There are two distinct ERs: ERα and ERβ, where ERα has a proliferative effect and ERβ acts as a repressor of ERα. Also PRs exist in two isoforms, as products of the same gene; where PRA lacks 164 amino acids from the N-terminal end of PRB (Oehler et al., 2000). PRB acts as transcriptional activator of progesterone target genes, while PRA has dual role which is cell- and promoter-specific. It can act as transcriptional factor or as repressor of PRB function (Vegeto et al., 1993).

In the present study, we have examined the mRNA levels of 11 estrogen- and progesterone-metabolizing enzymes (17β-HSD types 1, 2, 4, 7, 8 and 12, sulfatase, sulfotransferase, aromatase, AKR1C3 and AKR1C1) and steroid receptors (ERα, ERβ and PRAB) in specimens of ovarian endometriosis and control endometrium.

2. Materials and methods

2.1. Tissue samples

A total of 34 samples were collected: 24 of ovarian endometriomas and 10 of control endometrium, from women with *Uterus myomatosus* or *Myoma uterii* (Tables 1 and 2). The study was approved by the National Medical Ethics Committee of the Republic of Slovenia. RNA extraction, cDNA synthesis, real-time PCR and statistical analyses were performed as previously reported (Šmuc et al., 2007a,b). Expression analysis of 17β-HSD types 4, 8 and 12, AKR1C1 and AKR1C3 was performed on the 24 samples, and for another 9 genes on 16 samples of ovarian endometriomas.

Table 2
Patients details: control group

Sample	Age	Diagnosis	Phase of menstrual cycle
1	42	*U. myomatosus*	Non-proliferative
2	47	*M. uteri*	Late secretory
3	43	*U. myomatosus*	Early secretory
5	45	*U. myomatosus*	Proliferative
6	50	*U. myomatosus*	Early secretory
8	42	*M. uteri*	Late secretory
9	47	*U. myomatosus*	Proliferative
10	48	*M. uteri*	Proliferative
11	43	*U. myomatosus*	Early secretory
25	60	*M. uteri*	Atrophic endometrium

2.2. Western blotting

Aliquots from the protein fractions of ovarian endometriomas (isolated from the same samples as for RNA) were separated by SDS–PAGE. The proteins were transferred from the gels to membranes and incubated with 5% non-fat milk to avoid non-specifical binding. The membranes were then incubated overnight with primary antibodies: mouse anti-AKR1C3 (1:1000, Sigma–Aldrich, USA), mouse anti-PR-B (1:500, Santa Cruz, USA). The next day, the membranes were incubated with the secondary antibodies (anti-mouse peroxidase-conjugated, dilutions 1:3000 and 1:10,000, Jackson ImmunoResearch Laboratories Inc., USA) for 1 h. Supersignal West Pico Chemiluminiscence Substrate (Pierce Biotechnology) was used for the detection of the bound antibodies, according to the manufacturer instructions.

2.3. Immunohistochemistry

Formalin-fixed, paraffin-embedded samples containing ovarian endometriomas were dewaxed in xylene and rehydrated. The sections were incubated in H_2O_2 to block endogenous peroxidase. After antigen retrieval in sodium citrate buffer, sections were incubated with anti-AKR1C3 (1:500, Sigma–Aldrich, USA), anti-ERα (1:20, DakoCytomation, Denmark), or anti-PR-B (1:50, Santa Cruz, USA) antibodies. The peroxidase–antiperoxidase complex with DAB as substrate was used to detect bound antibodies.

3. Results and discussion

Aromatase is a key enzyme in local estrogen production (Noble et al., 1996) and aromatase inhibitors have proven to be beneficial for the treatment of endometriosis in some patients (Amsterdam et al., 2005). We saw that aromatase transcript levels were significantly higher ($p < 0.001$) in the endometriosis group, compared to normal (Fig. 1I) (Šmuc et al., 2007b), confirming previously published data (Noble et al., 1996; Bulun et al., 2005). The aromatase pathway also includes 17β-HSDs, which convert E1 to the highly active E2, and *vice versa*. In endometriotic tissue, all of the reductive 17β-HSDs were up-regulated. Although Zeitoun et al. (1998) have previously reported no differences in steady-state 17β-HSD type 1 mRNA between eutopic endometrial and endometriotic samples in different phases of the cycle, our published data (Šmuc et al., 2007a,b) show higher levels of type 1 transcript in ovarian endometriomas (Fig. 1A) ($p < 0.001$). Also, Dassen et al. (2007) recently reported up-regulation of 17β-HSD type 1 in deep infiltrating endometriosis. We have also shown higher levels of 17β-HSD type 7 in endometriotic tissue compared to normal (Šmuc et al., 2007a,b) (Fig. 1B) ($p < 0.001$). The expression of 17β-HSD type 12 has not yet been examined either in normal or diseased endometrium; here, we detected 17β-HSD type 12 mRNA in ovarian endometriosis and in normal endometrium tissue samples, and for the first time, we show that also 17β-HSD type 12 is up-regulated in the diseased endometrium (Fig. 1C) ($p = 0.0016$).

For oxidative 17β-HSDs, Zeitoun et al. (1998) have reported an absence of 17β-HSD type 2 mRNA transcripts in extra-ovarian endometriotic tissue with Northern blotting; however, our more sensitive study saw this transcript in both the control and the endometriosis group, although we did not detect any significant changes in the expression levels between these groups (Šmuc et al., 2007a,b) ($p > 0.05$) (Fig. 1D). In contrast, Matsuzaki et al. (2006) and Dassen et al. (2007) have shown lower expression of 17β-HSD type 2 in deep infiltrating endometriotic tissue. There have been no reports of decreased levels of 17β-HSD type 2 in ovarian endometriosis. For 17β-HSD types 4 and 8, we did not find significant differences in their expression levels (Fig. 1E and F), although Dassen et al. (2007) have reported a significant down-regulation of 17β-HSD type 4 in deep infiltrating endometriosis. However, at least to our knowledge, we are the