Expression of 20α-Hydroxysteroid Dehydrogenase mRNA in Human Endometrium and Decidua

TATSUYA NAKAJIMA, KATSUMI YASUDA, MIKIO NISHIZAWA*, HIDETAKA OKADA, TOMORI YOSHIMURA, SEIJI ITO* AND HIDEHARU KANZAKI

Department of Obstetrics and Gynecology, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi, Osaka 570-8507, Japan
*Department of Medical Chemistry, Kansai Medical University, 10-15 Fumizono-cho, Moriguchi, Osaka 570-8507, Japan

Abstract. Progesterone secreted from ovarian corpus luteum plays pivotal roles in endometrial differentiation, and local progesterone metabolism to regulate its concentration in endometrial tissues is essential for the successful implantation and maintenance of pregnancy. In this study, we evaluated the expression of mRNA for 20α-hydroxy-steroid dehydrogenase (20α-HSD), a key enzyme which converts progesterone to a biologically inactive metabolite, in human endometrial tissues and cultured endometrial stromal cells as well as decidua and chorionic tissues of early pregnancy. The level of 20α-HSD mRNA expression in secretory phase endometrium was significantly higher than that in proliferative phase endometrium and chorionic tissues. The expression level in decidual tissue was also significantly higher than that in chorionic tissue. In cultured endometrial stromal cells, 20α-HSD mRNA expression was slightly enhanced at a lower progesterone concentration of 0.01 μmol/l, and an increase in its expression was significantly suppressed at higher concentrations of 1 μmol/l or greater. No effect on the gene expression was seen in cultured endometrial stromal cells with various concentrations of 17β-estradiol. These results suggest that progesterone itself contributes to the regulation of local progesterone concentration through 20α-HSD levels in endometrial stromal cells at peri-implantation periods.

Key words: 20α-HSD, Progesterone, Chorion, Decidua, Endometrium

IT has been recognized that the most important role of progesterone in reproduction is to differentiate proliferative phase endometrium into secretory phase ones and then into decidua for blastocyst implantation and maintenance of pregnancy. Since progesterone is produced not only ovarian corpus luteum but also fetal trophoblasts, it is important to clarify the progesterone metabolism in the tissues in order to understand the local regulation of progesterone concentration.

The 20α-hydroxy-steroid dehydrogenase (20α-HSD) is an important enzyme that catalyzes the reaction of progesterone to 20α-hydroxyprogesterone, a biologically inactive form. In humans, it has been revealed that 3β-hydroxysteroid dehydrogenase (3β-HSD) purified from the liver possesses the enzyme activity of 20α-HSD [1]. DD1, which is also referred to as aldol-keto reductase (AKR) 1C1, is a member of the AKR superfamily [2]. Subsequently, complementary DNA (cDNA) encoding DD1 was cloned and identified as human 20α-HSD [3-6]. The amino acid sequences of human 20α-HSD has a much higher amino acid identity to other human AKRs; it is 98% identical to bile acid-binding protein (BABP)/DD2 [AKR1C2] [5]; 88% to prostaglandin F synthase (PGFS)/3α-HSD type 2 [AKR1C3] [7-9]; and 83% to DD4/chloride reductase/3α-HSD type 1 [AKR1C4] [4, 10, 11]. Therefore, it is very difficult to use cDNA probes or antibodies to distinguish human 20α-HSD from BABP, PGFS, and DD4 by Northern
or Western blot analysis.

Recently, we have succeeded in characterizing the highly homologous genes of 20α-HSD, BAPB, PGFS, and DD4, and then distinguished the expression of each gene by high-stringency polymerase chain reaction (PCR) with gene-specific primers [12]. Additionally, we revealed the expression of these genes in various human tissues by using this method, and found that 20α-HSD is expressed in the ovary, uterus, and placenta [12]. In the present study, we examined 20α-c-HSD mRNA expression in proliferative and secretory phase endometrium, decidua, and chorionic tissues by reverse transcription (RT) and high-stringency PCR with each gene-specific primer. We also examined the gene expression in cultured endometrial stromal cells with various doses of progesterone or 17β-estradiol.

This study was approved by Kansai Medical University ethics committee. The endometrial tissues were obtained from patients who underwent hysterectomy for myoma or adenomyosis. The endometrial phase was determined by histological findings. Human decidua and chorionic tissues were obtained from patients who selected termination of pregnancy and underwent dilatation and curettage at 7 to 9 weeks gestation. For the remaining experiments, the normal part of the ovary containing the corpus luteum was obtained from a non-pregnant patient who underwent an oophorectomy for a benign ovarian tumor. The tissues except for cell cultures were frozen in liquid nitrogen immediately and stored at −80°C until RNA extraction.

**Cell culture**

Endometrial stromal cells were purified from the proliferative phase endometrium and cultured as previously reported [13-15]. Briefly, tissue samples were washed with Phenol Red-free DMEM/Ham’s F-12 medium and minced into small pieces of less than 1 mm³. The tissues were incubated for 2 h at 37°C in Phenol Red-free DMEM/Ham’s F-12 medium containing 1 mg/ml collagenase and 0.005% DNase type I. After subsequent pipetting, the cell suspension was diluted with 2-volume Phenol Red-free DMEM/Ham’s F-12 medium and placed in a centrifugation tube for 10 min at unit gravity. The supernatant, excluding the lowermost 2 ml, was transferred into a new tube to collect suspended single cells. After repeating this procedure several times, the cell suspension was washed and used as a source of endometrial stromal cells. The viability, determined by dye exclusion, was more than 90%. Two million viable endometrial stromal cells were cultured in 75 cm² flasks in Phenol Red-free DMEM /Ham’s F-12 medium supplemented with 10% FCS, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37°C in humidified atmosphere of 5% CO₂ in air. The culture medium was replaced 30 min after plating to reduce epithelial cell contamination. After 1-2 passages when endometrial stromal cells were nearly confluent, cells were plated in 6-well plates. Endometrial stromal cells were cultured until confluent and the media were replaced with Phenol Red-free DMEM/Ham’s F-12 plus 10% FCS supplemented with various doses of progesterone (0.01, 0.1, 1,
10 and 100 μmol/l), 17β-estradiol (0.1, 1, 10, 100 and 1000 nmol/l) or ethanol (0.1% (v/v)) as a vehicle control. The cells were harvested on day 12 and were frozen in liquid nitrogen immediately and stored at −80°C until RNA extraction.

**RT-PCR**

Total RNA was extracted and prepared according to published methods [16]. Ten micrograms of total RNA was reverse transcribed by Superscript II reverse transcriptase (200 units) with random hexamers (10 ng) in 20 μl of reaction mixture, containing 1×first strand buffer, DTT (10 mmol/l), dNTP (1 mM), and RNase inhibitor (15 units). The reaction was carried out at 47°C for 60 min. After reverse transcription, RNase H (2 units) was added to the reaction mixture. It was incubated at 37°C for 10 min and the volume was adjusted up to 100 μl with Tris-EDTA (TE) buffer, containing Tris (10 mmol/l, pH 7.5) and EDTA (1 mmol/l). The first strand cDNA was prepared by phenol/chloroform extraction and ethanol precipitation as follows: 100 μl of phenol and chloroform mixture (1:1) was added to the reaction mixture. After vortexing, it was centrifuged at 12,000 × g for 3 min and the aqueous phase was saved. As a carrier, glycogen (40 μg) was added, as well as ammonium acetate (2 mol/l) and 350 μl of ethanol. The mixture was placed at −20°C for 15 min and centrifuged at 12,000 × g for 20 min. The supernatants were removed and the pellets were washed with 1 ml of 70% ethanol and dried. The mixture was dissolved in 50 μl of TE buffer. As a template, 0.4 μg of the cDNA was used for the PCR.

We performed high-stringency PCR with 20α-HSD gene-specific primers (forward and reverse primers, 50 pmol each) in 100 μl of the reaction mixture, containing 1×Gene Taq universal buffer, dNTP (0.1 mmol/l), and Gene Taq DNA polymerase (1.7 units). The step-down PCR protocol applied was as follows: 10 cycles of (94°C, 1 min; 72°C, 3 min), 15 cycles of (94°C, 1 min; 65°C, 2 min; 72°C, 30 sec), and 5–25 cycles of (94°C, 1 min; 60°C, 2 min; 72°C, 30 sec). The linearity of DNA amplification and the correctness of the products were confirmed according to published methods [12]. The same PCR protocol with ribosomal protein S26 (S26) gene-specific primers was applied in every samples studied as an internal standard. The gene-specific primers are shown in Table 1.

**Quantification and statistical analysis**

Each amplified DNA band was quantified by densitometry of agarose gel under an ultraviolet (UV) transilluminator (FluorChem, Alpha Inotech, San Leandro, CA, USA). For normalization, we calculated the ratios between each intensity of DNA band corresponding to 20α-HSD and that corresponding to S26. In the cell culture experiment, each sample value was divided by the values of the control (vehicle). The data were expressed as mean ± SD, and were analyzed by one-factor ANOVA and a Bonferroni/Dunn test using the StatView software program (Abacus Concepts, Inc., Berkeley, CA, USA). Statistical significance was set at P<0.05.

**Results**

DNA bands amplified by high-stringency RT-PCR with 20α-HSD gene-specific primers were observed in proliferative and secretory phase endometrium, decidua, and chorionic tissues (Fig. 1A). An amplified DNA band was also observed in the ovary (Fig. 1A). The ratios of the 20α-HSD to S26 in proliferative and secretory phase endometrium, decidua, and cho-

### Table 1. Primers for high-stringency polymerase chain reaction (PCR)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20α-HSD/DD1 [AKRIC1]</td>
<td>DD1-F2</td>
<td>ACCAAATGCGCAATTGAAGCT</td>
</tr>
<tr>
<td></td>
<td>DD1-R3</td>
<td>TAAGGATAGAGGTCAAACATAAT</td>
</tr>
<tr>
<td>S26</td>
<td>S26-F</td>
<td>GGTCCGTGCCTCAGAGATGACAAA</td>
</tr>
<tr>
<td></td>
<td>S26-R</td>
<td>GAACCTCAGCTCCTACATGGCCCT</td>
</tr>
</tbody>
</table>

Sets of gene-specific forward (F, upper) and reverse (R, lower) primers are shown.
The expression of 20α-HSD mRNA in human reproductive tissues. A: The upper and lower panels show 2.0% agarose gel electrophoresis of high-stringency PCR products with 20α-HSD and S26 specific primers, respectively. The products derived from proliferative and secretory phase endometrium, decidua, and chorion are shown in lanes 1 to 5, lanes 6 to 10, lanes 11 to 15, and lanes 16 to 20, respectively. In lane 21, the product derived from the ovary is shown. B: A comparison of 20α-HSD mRNA expression in proliferative and secretory phase endometrium, decidua, and chorion. The ratios of the quantified values to the corresponding values of S26 were calculated for normalization. The data are expressed as the means (open boxes) ± SD (bars). Five samples were used. An asterisk indicates a statistically significant difference (P<0.05). Abbreviations: P: proliferative phase endometrium, S: secretory phase endometrium, D: decidua, C: chorion, O: ovary.

In cultured endometrial stromal cells, 20α-HSD DNA bands amplified by RT-PCR were observed in every samples studied (Figs. 2A, 3A). In the various doses of 17β-estradiol added group, the expression level of 20α-HSD was not significantly changed on day 12 (Fig. 2B). The expression level of 20α-HSD mRNA was slightly enhanced at a lower progesterone concentration of 0.01 μmol/L, and an increase in its expression was significantly suppressed at higher concentrations of 1 μmol/L or greater. (P<0.01, Fig. 3B).

The DNA bands amplified by high-stringency RT-PCR with S26 gene-specific primers were observed in each tissue type studied (Figs. 1A, 2A, 3A). The S26 expression level was not statistically significant in any of the samples.

Discussion

In this study, we were the first to report the mRNA expression levels of 20α-HSD, which catalyzed the reaction that converts progesterone to an inactive form, in human endometrium, decidua, chorion and cultured endometrial stromal cells. And it revealed that 20α-HSD mRNA expression levels in secretory phase endometrium were significantly higher than those in the proliferative phase endometrium. Post-translational modification of 20α-HSD does little to affect its enzymatic activity [17], therefore, the enzymatic activity of 20α-HSD is mainly related to its mRNA expression levels. Previously, Pollow et al. reported that the enzymatic activity of 20α-HSD
in secretory phase endometrium is higher than that in proliferative phase endometrium [18]. Our results confirm their findings at the gene expression level.

In rat corpus luteum, the expression of 20α-HSD was decreased by progesterone [19] and prolactin [20], whereas the expression was increased by prostaglandin F2α (PGF2α) [21]. We previously reported that the promoter activity of human 20α-HSD was increased by PGF2α and oxytocin in a model experiment using porcine granulosa cells [12]. In this study, we observed that the level of 20α-HSD mRNA was decreased in cultured human endometrial stromal cells when progesterone was added to the medium at the concentration of 1 μmol/l or greater. This may contribute to increase progesterone level in endometrial tissues when implanting trophoblasts get to producing progesterone. In decidua, prolactin secreted by decidualized stromal cells may also contribute to decrease 20α-HSD. Yasui et al. reported that prolactin-releasing peptide produced by the human decidua did not affect decidual prolactin secretion due to a lack of the receptor [22]. Regulation of decidual prolactin secretion remains unknown. In human endometrium, PGF2α levels are known to increase during the secretory phase [23], and this may cause an increase of 20α-HSD mRNA expression in the secretory phase endometrium to scavenge local progesterone towards menstruation. We also observed that the level of 20α-HSD mRNA was slightly increased in cultured human endometrial stromal cells when progesterone was added to the medium at a concentration of 0.01 μmol/l (= 10 nmol/l). In proliferative phase, human serum progesterone concentration is 0.3–3.0 nmol/l, whereas in secretory phase, its level rises up to 19.0–45.0 nmol/l [24].
This does not conflict with our result that the expression level of 20α-HSD mRNA in secretory phase endometrium was higher than that in proliferative phase endometrium. In cultured human endometrial stromal cells, the regulation mechanism of 20α-HSD mRNA expression remains unknown. However, progesterone may variously contribute to the regulation of 20α-HSD mRNA expression depending on its concentration level.

Interestingly, 20α-HSD mRNA expression was found in the chorionic tissues which produce progesterone by themselves, although the level of expression was significantly lower than that in secretory phase endometrium and decidua. The reason for 20α-HSD expression in chorionic tissues remains unknown, but the enzyme may regulate the level of progesterone inside the placenta and fetal circulation.

In conclusion, our present results indicate that, in human endometrium, progesterone affects the mRNA expression of 20α-HSD, a key enzyme of local progesterone metabolism, and that this may be important to regulate progesterone concentration in the endometrium for successful implantation and maintenance of pregnancy.

**Acknowledgements**

We thank Fusco Pharmaceutical Industries, Ltd. and Kurabo Industries, Ltd. Biomedical Department for the oligonucleotide synthesis. This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas and Scientific Research (B: No. 12470350) and (C: No. 10671577) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**References**


