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Effects of testosterone on PPARγ and P450arom expression in polycystic ovary syndrome patients and related mechanisms

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Abstract. – OBJECTIVE: Polycystic ovary syndrome (PCOS) causes ovulation disorder and infertility in female patients. This study recruited PCOS patients and adopted testosterone therapy to analyze its effect on expression of nuclear peroxisome proliferation activated receptor γ (PPARγ), and cytochrome P450 aromatase (P450arom) in ovary granular cells of patients.

PATIENTS AND METHODS: PCOS and non-PCOS patients were treated with testosterone at 1.0 nmol/L, 10 nmol/L. qRT-PCR was used to detect the expressions of PPARγ and P450arom while Western blot was performed for the evaluation of protein expression.

RESULTS: In the experimental group, the level of PPARγ mRNA was significantly elevated whilst the expression of P450arom was statistically decreased ($p < 0.05$). The treatment of 10 nmol/l testosterone significantly elevated PPARγ mRNA and protein expression, and decreased P450arom expression ($p < 0.05$). The level of PPARγ was positively correlated with testosterone concentration, and was negatively correlated with P450arom expression ($p < 0.05$).

CONCLUSIONS: Testosterone causes hyperandrogenism microenvironment of PCOS patients, with a correlated increase of PPARγ and reduction of P450arom.

Key Words:
Testosterone, Polycystic ovary syndrome, PPARγ mRNA, P450arom.

Introduction

Polycystic ovary syndrome (PCOS) manifests as ovulation disorder, and represents the major cause of women menopause or female infertility. At hormonal level, hyperandrogenism and hyperinsulinemia were developed in PCOS patients, with a rising level of follicle stimulating hormone (FSH) or luteinizing hormone (LH). Abundant small ovary follicles without matured ones were found in PCOS patients due to the dysfunction of the hypothalamus-pituitary-ovary axis, and disorder of follicle selection or arresting. PCOS patients are more susceptible for metabolic disorders including type 2 diabetes and hypertension[1-3]. It has been demonstrated that, during the pathogenesis of various diseases, hyperandrogenism primarily contributed to the disorder of glucose or lipid metabolism in PCOS women[4].

Nuclear peroxisome proliferation activator receptor γ (PPARγ) belongs to nuclear receptor superfamily, and regulates the occurrence and development of chronic metabolic disorders including diabetes, metabolic syndrome or hypertension[5,6]. In ovary tissues, PPARγ is mainly expressed in ovary granular cells at the development stage, and regulate estrogen secretion and ovary function under the direction of LH peak and low values[7]. Cytochrome P450 aromatase (P450arom) is highly expressed in granular cells, and can lead to hyperandrogenism once being suppressed for normal transformation[8]. In this study, the effect of testosterone on PPARγ and P450arom in PCOS patients was determined.

Patients and Methods

Patients Information

A total of 30 PCOS patients (25-40 years old, average age = 29.2 ± 3.5 years) who were admitted to Jinan Maternity and Child Care Hospital from October 2015 to September 2016 were recruited. All patients were for the compliance with PCOS diagnostic criteria: infrequent menstruation; infertility; hairiness; obesity; LH in early phase ovary or after drug retraction/FSH > 3 and/or above normal testosterone level; enlargement of bilateral ovary; More than 10 large follicles

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(> 10 mm diameter) in unilateral ovary. Another 30 non-PCOS patients (aging between 25 and 35 years, average age = 28.5 ± 3.1 years) who were characterized by normal menstrual cycle and ovulation were recruited. No significant difference existed regarding age, height or body weight between two groups, which were thus comparable ($p > 0.05$).

Inclusive criteria: No treatment of hormone drugs within 3 months; no malignant lesion occurred in ovary or uterus; no severe liver/renal disease.

Exclusive criteria: Complication of uterus body or cervical inflammation; other gynecology disease or endometrial disorder; long-term user of hormonal drugs; complication with uterus, ovary or oviduct.

The study protocol was approved by the Research Ethics Committee of Jinan Maternity and Child Care Hospital, and all patients signed the informed consent before the start of investigation.

Reagents

DMEM medium, streptomycin/penicillin and fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA); q-PCR test kit, reverse transcription cDNA test kit (Invitrogen, Waltham, MA, USA); Biometra T1 PCR cycler Bio-Rad (Hercules, CA, USA).

Granular Cell Separation

Follicles were collected via virginal route for extracting follicular fluid and were cultured *in vitro*. Follicular fluid was centrifuged and placed in PBS-Percoll mixture. Upper layer cells were removed in NH_4Cl for further centrifugation, and suspensions of ovary granular cells were filtered and obtained. Cells were re-suspended in DMEM containing 10% FBS and streptomycin/penicillin for 48 h culture. Cells were, then, fixed in paraformaldehyde and stored at -80°C.

Granular Cell Culture

Experimental group A and B were treated with 1 nmol/L and 10 nmol/L testosterone (N=10). Group C was maintained by using normal cell culture medium (N=10). Cells were repeatedly rinsed and mixed with TRIzol for -80°C storage.

qRT-PCR for PPARγ and P450arom Expression in Ovary Granular Cells

According to the instruction of the PCR kit, PCR mixture was prepared including 0.1 ml cDNA, and 1 ml PCR buffer in 1 ml pre-mix. The reaction plate was sealed by the membrane, 20 µl reaction buffer was added for centrifugation. PCR conditions were: 95°C 5 min, followed by 40 cycles each of 95°C 5 s, and 60°C 30 s (Table I).

Western Blot for PPARγ and P450aroma Protein Expression in Ovary Granular Cells

Cells were rinsed and lysed for further mixture with 0°C RIPA lysis buffer. The mixture was centrifuged, and the supernatant was collected and stored at -80°C. Proteins were boiled at 100°C. Proteins were separated by electrophoresis and were transferred to the membrane, which was incubated with primary antibody (1:100 dilution) for 2 h. After rinsing, goat anti-rabbit secondary antibody was added for 1 h incubation. After development and exposure, the bands in the membrane were scanned and analyzed.

Data Processing

SPSS17.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data processing and data were presented as mean±standard deviation (SD). Enumeration data were presented by chi-square analysis, whilst measurement data were analyzed by Student *t*-test. The correlation between PPARγ mRNA or P450arom and testosterone was analyzed by Spearman approach. A statistical significance was defined when $p < 0.05$.

Table I. Primer synthesis.

Gene		Temp (°C)	Length (bp)
PPARγ mRNA	5'-CAGGAGCAGAGCAAAGA-3' 5'-GGACTCAGGGTGGTTCA-3'	60	274
P450arom	5'-CAGCGGTCTCCCTTGATA-3' 5'-TCTTCTCGGCATTTCTCC-3'	60	314
GAPDH	5'-GCACCGTCAAGGCTGAGAAC-3' 5'-TGGTGAAGACGCCAGGGA-3'	60	580

Results

PPARγand P450arom Expression in Ovary Granular Cells Before Treatment

Expression of PPARγ mRNA and P450arom in patients with ovary granular cells was measured. Compared to control group, the expression of PPARγ was significantly increased in experimental groups, with statistical reduction of P450aroma expression ($p < 0.05$, Figure 1).

PPARγ and P450arom Gene Expression in Ovary Granular Cells After Treatment

We further measured the contents of PPARγ mRNA and P450arom in PCOS patients after testosterone treatment. The elevation of PPARγ and the decrease of P450arom levels were presented in a dose of testosterone-dependent manner ($p < 0.05$, Figure 2).

PPARγ and P450arom Protein Expression in Ovary Granular Cells

We also tested the expressions of PPARγ and P450arom in ovary granular cells after testosterone treatment. In the group of 10 nmol/l testosterone, PPARγ protein expression was significantly up-regulated and P450arom level declined compared to the group of control or low dose of testosterone ($p < 0.05$, Table II, Figure 3).

Correlation Between PPARγ and P450arom Expression in Ovary Granular Cells As Well As Testosterone

In ovary granular cells, PPARγ mRNA expression was positively correlated with testosterone expression ($r = 0.640$, $p < 0.01$) and was negatively related to P450arom expression ($r = -0.738$, $p < 0.01$).

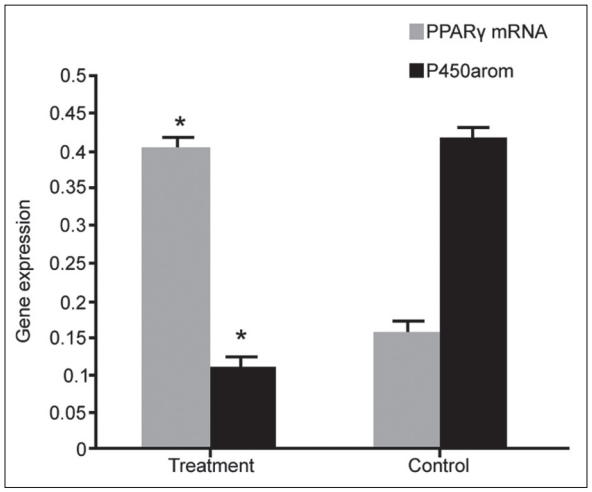

Figure 1. Expression of PPARγ mRNA and P450arom in ovary granular cells. *, $p < 0.05$ compared to control group.

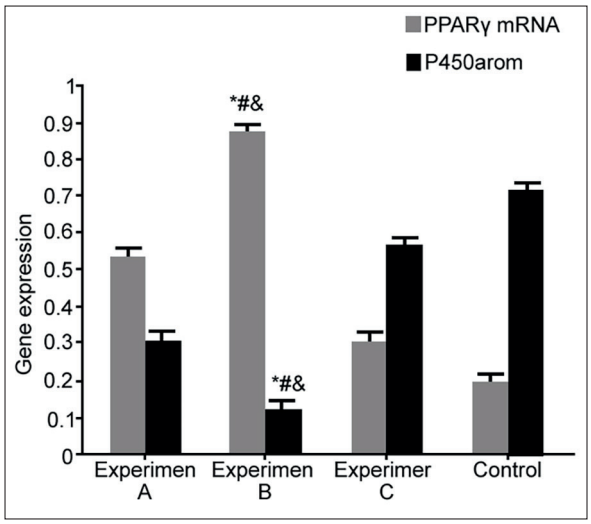

Figure 2. PPARγ mRNA and P450arom gene expression in ovary granular cells after testosterone intervention. *, $p < 0.05$ compared to control group; #, $p < 0.05$ compared to experimental group A; &, $p < 0.05$ compared to experimental group C.

Discussion

PCOS represents a type of common endocrine disorder in clinics. The fertile period is largely determined by total depository of oocytes and their apoptotic rate[9-11]. Hyperandrogenism belongs to a kind of unique endocrine manifestation of PCOS and severely affects normal ovulation, leading to infertility of PCOS women[12]. P450arom activity was closely correlated with androgen/estrogen transition. As the enzyme activity was suppressed, androgen cannot be effectively

Table II. Protein expression of PPARγ and P450arom in ovary granular cells after testosterone.

Group	N	PPARγ	P450arom
Experiment	30		
A	10	1.932 ± 0.004	1.376 ± 0.003
B	10	2.549 ± 0.008*#&	1.034 ± 0.002*#&
C	10	1.378 ± 0.004	1.668 ± 0.004
Control	30	1.253 ± 0.002	2.476 ± 0.011

Note: *, $p < 0.05$ compared to control group; #, $p < 0.05$ compared to experimental group A; &, $p < 0.05$ compared to experimental group C.

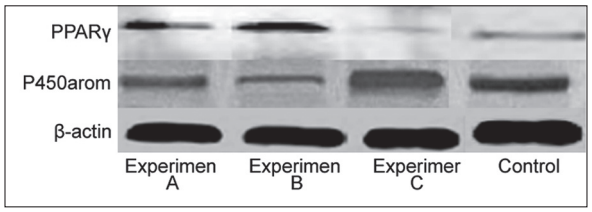

Figure 3. PPARγ and P450arom protein expression in ovary granular cells after testosterone treatment.

transformed into estrogen, which resulted in focal hyperandrogenism[9,10]. PPARγ expression is positively correlated with follicular testosterone concentration and free androgen index of PCOS patients[13]. Liang et al[14] generated PCOS mice and indicated that P450arom was the downstream factor of TGF-β/Smads signal transduction pathway. In human liver/renal tissues, agonist of PPAR can decrease Smad2 phosphorylation level and exert certain inhibitory effects on TGF-β/Smads signal transduction pathway. In ovary granular cells, however, the interaction between PPAR and P450arom is still unclear.

In this work, we found that PPARγ mRNA was up-regulated and P450arom was down-regulated in PCOS patients and ovary granular cells. Consistently, a previous study[15] showed the statistical correlation between P450aroma gene polymorphism and PCOS pathogenesis. The hyperandrogenism occurred in PCOS women, mainly due to the alternation of P450arom substrate and inactivity. Furthermore, the up-regulation of PPARγ was exhibited in ovary granular cells and PCOS patients, as in agreement with our study.

Faut et al[16] investigated the correlation between antral follicle apoptosis in PCOS patients and PPARγ expression, and validated that PPARγ affected pregnant outcome via mediating follicular growth. Additionally, abnormal aromatization of P450arom affects follicular development. In this study, the concentrations of 1 nmol/l and 10 nmol/l testosterone were used for the evaluation of the effect of testosterone on PPARγ and P450arom from ovary granular cells of recruited patients. Of note, high dose of 10 nmol/l testosterone remarkably increased the level of PPARγ, with down-regulation of P450arom expression, compared with that of control or low dose of testosterone. The genomic study showed up-regulation of PPARγ mRNA in ovary tissues of PCOS patients[17]. Evidence on PCOS mouse model showed that the hyperandrogenism microenvironment caused by exogenous interference can change PPARγ mRNA expression level in ovary granular cells of PCOS mice, leading to altered methylation level[18], which was in line with our finding on positive correlation between testosterone and PPARγ. Hyperandrogenism can induce phenotypic changes of PPARγ in ovary granular cells to certain extents and is frequently accompanied by ovary dysfunction[19]. The clustered analysis also indicated the correlation between PPARγ gene polymorphism and PCOS onset and occurrence, which was closely associated with hyperandrogenism[20]. Mallqueo et al[21] utilized P450arom inhibitor as a continuously exogenous stimulus to study its role on PCOS-induced hyperandrogenism model of mice. Assays showed that once P450arom gene activity was suppressed, it can directly participate in the onset and progression of PCOS-related hyperandrogenism, which combined with our data on negative correlation between P450arom and hyperandrogenism, suggesting the inhibitory effect of P450arom on hyperandrogenism[21], which confirmed that the decreased activity of P450aroma was associated with the androgen-estrogen transition. As multiple factors were identified in the regulation of PCOS[22], and in the future, precise assay for the study of PPARγ and P450arom, combined with treatment such as metformin, may provide novel idea and strategy for the therapy of PCOS.

Conclusions

Our data described the behavior of PPAR mRNA and of cytochrome P450aroma before and after different doses of testosterone in ovary granular cells and PCOS women. Testosterone significantly elevated the PPARγ expression while down-regulated the level of P450arom in both ovary granular cells and PCOS patients. PPARγ was positively correlated with testosterone but negatively related to P450arom expression.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Glintborg D, Andersen M. An update on the pathogenesis, inflammation, and metabolism in hirsutism and polycystic ovary syndrome. Gynecol Endocrinol 2010; 26: 281-296.

2) Glintborg D. Endocrine and metabolic characteristics in polycystic ovary syndrome. Dan Med J 2016; 63. pii: B5232.
3) Yin J, Gao Z, He Q, Zhou D, Guo Z, Ye J. Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. Am J Physiol Endocrinol Metab 2009; 296: E333-342.
4) Maciel GA, Baracat EC, Benda JA, Markham SM, Hensinger K, Chang RJ, Erickson GF. Stockpiling of transitional and classic primary follicles in ovaries of women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004; 89: 5321-5327.
5) Sato H, Sugai H, Kurosaki H, Ishikawa M, Funaki A, Kimura Y, Ueno K. The effect of sex hormones on peroxisome proliferator-activated receptor gamma expression and activity in mature adipocytes. Biol Pharm Bull2013; 36: 564-573.
6) Qu F, Wang FF, Yin R, Ding GL, El-Prince M, Gao Q, Shi BW, Pan HH, Huang YT, Jin M, Leung PC, Sheng JZ, Huang HF. A molecular mechanism underlying ovarian dysfunction of polycystic ovary syndrome: hyperandrogenism induces epigenetic alterations in the granulosa cells. J Mol Med (Berl) 2012; 90: 911-923.
7) San-Millan JL, Escobar-Morreale HF. The role of genetic variation in peroxisome proliferator-activated receptors in the polycystic ovary syndrome (PCOS): an original case-control study followed by systematic review and meta-analysis of existing evidence. Clin Endocrinol (Oxf) 2010; 72: 383-392.
8) Garcia Barrado MJ, Blanco EJ, Carretero Hernandez M, Iglesias Osma MC, Carretero M, Herrero JJ, Burks DJ, Carretero J. Local transformations of androgens into estradiol by aromatase P450 is involved in the regulation of prolactin and the proliferation of pituitary prolactin-positive cells. PLoS One 2014; 9: e101403.
9) Koika V, Marioli DJ, Saltamavros AD, Vervita V, Kougiannis KD, Adonakis G, Decavalas G, Georgopoulos NA. Association of the Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma2 with decreased basic metabolic rate in women with polycystic ovary syndrome. Eur J Endocrinol 2009; 161: 317-322.
10) Berhouma R, Kouidhi S, Ammar M, Abid H, Ennafaa H, Benammar-Elgaaied A. Correlation of peroxisome proliferator-activated receptor (PPAR-gamma) mRNA expression with Pro12Ala polymorphism in obesity. Biochem Genet 2013; 51: 256-263.
11) Chen Q, Sun X, Chen J, Cheng L, Wang J, Wang Y, Sun Z. Direct rosiglitazone action on steroidogenesis and proinflammatory factor production in human granulosa-lutein cells. Reprod Biol Endocrinol 2009; 7: 147.
12) Bas D, Abramovich D, Hernandez F, Tesone M. Altered expression of Bcl-2 and Bax in follicles within dehydroepiandrosterone-induced polycystic ovaries in rats. Cell Biol Int 2011; 35: 423-9.
13) Froment P, Touraine P. Thiazolidinediones and fertility in polycystic ovary syndrome (PCOS). PPAR Res 2006; 2006: 73986.
14) Liang N, Xu Y, Yin Y, Yao G, Tian H, Wang G, Lian J, Wang Y, Sun F. Steroidogenic factor-1 is required for TGF-beta3-mediated 17beta-estradiol synthesis in mouse ovarian granulosa cells. Endocrinology 2011; 152: 3213-3225.
15) Fan F, Li Y, Duan X, Zhao T, Pan D, Chen H. Rosiglitazone attenuates activation of human Tenon's fibroblasts induced by transforming growth factor-beta1. Graefes Arch Clin Exp Ophthalmol 2012; 250: 1213-1220.
16) Faut M, Elia EM, Parborell F, Cugnata NM, Tesone M, Motta AB. Peroxisome proliferator-activated receptor gamma and early folliculogenesis during an acute hyperandrogenism condition. Fertil Steril 2011; 95: 333-337.
17) Lebbe M, Woodruff TK. Involvement of androgens in ovarian health and disease. Mol Hum Reprod 2013; 19: 828-837.
18) Lazaros L, Xita N, Hatzi E, Takenaka A, Kaponis A, Makrydimas G, Sofikitis N, Stefos T, Zikopoulos K, Georgiou I. CYP19 gene variants affect the assisted reproduction outcome of women with polycystic ovary syndrome. Gynecol Endocrinol 2013; 29: 478-482.
19) Guan Y. Peroxisome proliferator-activated receptor family and its relationship to renal complications of the metabolic syndrome. J Am Soc Nephrol 2004; 15: 2801-2815.
20) Hao CF, Zhang N, Qu Q, Wang X, Gu HF, Chen ZJ. Evaluation of the association between the CYP19 Tetranucleotide (TTTA)n polymorphism and polycystic ovarian syndrome (PCOS) in Han Chinese women. Neuro Endocrinol Lett 2010; 31: 370-374.
21) Maliqueo M, Sun M, Johansson J, Benrick A, Labrie F, Svensson H, Lonn M, Duleba AJ, Stener-Victorin E. Continuous administration of a P450 aromatase inhibitor induces polycystic ovary syndrome with a metabolic and endocrine phenotype in female rats at adult age. Endocrinology 2013; 154: 434-445.
22) Medina G, Maliqueo M, Crisosto N, Echiburu B, Sir-Petermann T, Lara HE. Metformin increases norepinephrine transporter expression in placenta of patients with polycystic ovary syndrome. Eur Rev Med Pharmacol Sci 2017; 21: 3482-3489.