Restored Plasma Anandamide and Endometrial Expression of Fatty Acid Amide Hydrolase in Women With Polycystic Ovary Syndrome by the Combination Use of Diane-35 and Metformin

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ABSTRACT

Purpose: Polycystic ovary syndrome (PCOS) is a metabolic and endocrinal disorder affecting a number of women of reproductive age. We aimed to reveal the correlation between the endocannabinoid system and PCOS, which may provide a new therapeutic target for PCOS treatment.

Methods: Serum levels of anandamide and 2-arachidonoylglycerol andexpression of cannabinoid receptors and fatty acid amide hydrolase (FAAH) in the endometrium were compared between women with PCOS and infertile women without PCOS, as well as women with PCOS before and after treatment with Diane-35 and metformin. Cannabinoid receptors and FAAH in the endometrium were stained using the immunohistochemical method. Results were analyzed by calculating integrated optical density.

Findings: Plasma anandamide was increased significantly in women with PCOS compared with infertile women without PCOS. Treatment with Diane-35 and metformin reversed this increase in women with PCOS. No significant difference in 2-arachidonoylglycerol was observed between the infertile women with or without PCOS. The women with PCOS had lower endometrial expression of FAAH compared with infertile women without PCOS, whereas no significant difference in endometrial expression of cannabinoid receptors was observed between the women with PCOS and infertile women without PCOS. We found that after treatment with Diane-35 and metformin, FAAH expression tended toward a significant increase compared with women before the treatment.

Implications: Endocannabinoid system may be involved in the progression of PCOS, and serum anandamide could serve as a potential biomarker of clinical diagnosis of PCOS. (*Clin Ther*. 2017;39:751–758)

Key words: cannabinoid receptors, CB1, endocannabinoids, fatty acid amide hydrolase, FAAH, polycystic ovary syndrome, PCOS, metformin.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrinal and metabolic disorder, which may result from the interactions between genetic and environmental factors. Currently, PCOS is the most common cause of female infertility, leading to high risks of miscarriage, pre-eclampsia and gestational diabetes.[1] It is usually diagnosed by hyperandrogenism, chronic oligo and/or anovulation, and ultrasound morphology of the ovaries.[2] Not only sex hormones are altered in PCOS patients but also several metabolic complications such as obesity, diabetes mellitus, and insulin resistance may coexist with PCOS.[3]

Recently, dysregulation of endocannabinoid system (ECS) has been proposed to be related to the metabolic disturbances of PCOS[4,5] because of its key role in energy homeostasis. It was reported that increased levels of endocannabinoids and G-protein-coupled cannabinoid receptors (CB1) may be associated with obesity.[6] Regarding high prevalence of obesity in PCOS cases, disruption in ECS may be a potential

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factor in the development and progression of PCOS. Besides CB1, CB2, several bioactive lipids (the endocannabinoids), and enzymes together compose the ECS. N-arachidonoylethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are the two mostly studied endocannabinoids. Monoacylglycerol lipase (MAGL) mainly participates in the hydrolysis of 2-AG and fatty acid amide hydrolase (FAAH) is the principal catabolic enzyme for AEA.[7,8] In 2015, Juan CC *et. al* indicated that higher levels of AEA and 2-AG and elevated expressions of CB1 may be positively correlated with insulin resistance in women with PCOS.[5] Although a previous study suggested that no association was shown between endocannabinoid receptor 1 gene variations and metabolic complications in women with PCOS,[9] Jędrzejuk D *et. al* debated that women with endocannabinoid receptor 1 gene variations showed a higher risk of PCOS.[10]

However, better knowledge of the association between ECS and PCOS may provide a new method in clinical treatment of PCOS or reduce the metabolic complications in women with PCOS. Therefore, in this study, we compared the serum levels of AEA and 2-AG and the expressions of CB1 and FAAH between women with PCOS and infertile women without PCOS and also between women with PCOS before and after treatment with Diane-35 and metformin.

METHODS

Subjects

Thirty women (aged 23–33 years) diagnosed with PCOS participated as the experimental group. Thirty women (aged 24–34 years) diagnosed with infertility without PCOS were included as control group. Diagnosis of PCOS was based on the Rotterdam diagnostic criteria,[11] including oligoovulation or anovulation, hyperandrogenism, and polycystic ovaries on pelvic ultrasonography. More than 12 follicles with diameter of each follicle <10 mm were present in the enlarged ovary. Diseases leading to hyperandrogenism, such as androgenic tumor, congenital adrenal hyperplasia, and Cushing syndrome were distinguished from PCOS cases. Anovulation resulting from other complications, such as hyperprolactinemia, premature ovary failure, hypothalamic amenorrhea, and thyroid dysfunction, was excluded from PCOS. None of the subjects had undergone hormone therapy, radiotherapy, or operations on the uterus and ovaries. Smokers and alcoholic subjects were excluded. No subjects were using oral contraceptives or any medications affecting metabolism of lipid and glucose or liver function. All subjects signed the written informed consents before participation. The study was approved by Reproductive Center of Second Hospital of Hebei Medical University.

Blood and Endometrial Biopsies Collection

Patients with PCOS had been treated with Diane-35 (once per day) and metformin (500 mg, twice per day) for 3 months. All blood and endometrial biopsies were collected once in the infertile women without PCOS and twice in the women with PCOS as before and after treatment. Endometrial biopsies were obtained from all individuals using an endometrial suction curette (Pipet Curet; CooperSurgical, Trumbull, Connecticut) under an approved Human Investigations Committee protocol. All biopsies were the superficial layers of an area of the uterine cavity.

Measurements

For each subject, 5.0-mL blood samples were collected under fasting condition early in the morning on days 2 to 4 before menses in an EDTA anticoagulation tube. Serum was obtained after centrifugation at 3000 rpm, 4°C for 10 minutes, and stored at −80°C until analyses. Serum AEA and 2-AG levels were assessed. Briefly, 1 mL serum was incubated on ice for 15 minutes after adding 10 μL methanol internal standard solution. The solution was then mixed with methylbenzene and centrifuged at 4000 rpm, 4°C for 10 minutes. The upper organic phase was kept and dried under N_2 gas, and suspended with 500 μL water-methanol solution (1:3, v/v). Liquid chromatography-electrospray ionization-mass spectrometry (Agilent LC-MSD 1100 series; Agilent, Santa Clara, California) was used for analysis of AEA and 2-AG and isotope dilution was used for quantification, as described previously.[12]

Immunohistochemistry

Endometrial biopsies were obtained by curettage after human chorionic gonadotropin test. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. After deparaffinization, slides were placed in citric acid buffer and incubated in boiling water bath for 10 minutes for antigen retrieval. Then