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RESEARCH ARTICLES

Brown adipose tissue activation by rutin ameliorates polycystic ovary syndrome in rat☆

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Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrinopathy that is characterized by anovulation, hyperandrogenism and polycystic ovary. However, there is a lack of effective treatment for PCOS at present because the pathologic cause of PCOS has not been elucidated. Although it has been known that brown adipose tissue transplantation ameliorates PCOS by activating endogenous BAT, BAT transplantation is not applicable in clinic. Therefore, BAT activation with natural compound could be an effective treatment strategy for PCOS patients. Here, we found that 3 weeks of rutin (a novel compound for BAT activation) treatment increased BAT activation, thereby it improved thermogenesis and systemic insulin sensitivity in dehydroepiandrosterone (DHEA)-induced PCOS rat. In addition, the expression levels of ovarian steroidogenic enzymes such as P450C17, aromatase, 3β-HSD, 17β-HSD and STAR were up-regulated in rutin-treated PCOS rat. Furthermore, acyclicity and the serum level of luteinizing hormone were normalized, and a large number of mature ovulated follicle with a reduction of cystic formation were observed in PCOS rat after rutin treatment. Finally, rutin treatment surprisingly improved fertility and birth defect in PCOS rat. Collectively, our results indicate that rutin treatment significantly improves systemic insulin resistance and ovarian malfunction in PCOS, and our findings in this study provide a novel therapeutic option for the treatment of PCOS by activating BAT with rutin.
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Keywords: Polycystic ovary syndrome; Brown adipose tissue; Rutin; Insulin resistance; Fertility

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common reproductive endocrine disorder affecting women of reproductive age. Its prevalence varies from 9 to 18% depending on the diagnostic criteria used and population assessed [1–3]. Women with PCOS are characterized by hyperandrogenism, reproductive polycystic ovaries and chronic anovulation. In addition, PCOS is associated with higher risk of insulin resistance, dyslipidemia, obesity, Type 2 diabetes (T2D) as well as cardiovascular diseases [4–6]. According to the widely accepted Rotterdam Consensus, women can be diagnosed with PCOS when they follow at least any two of three features: androgen excess, ovulatory dysfunction and polycystic ovary appearance on ultrasound after excluding other causes of these abnormalities [7,8]. The etiology of PCOS is complex, and several environmental and genetic factors may result in the development of PCOS. Nonetheless, the potential cellular mechanisms for the development of PCOS remain to be elucidated.

Insulin resistance (IR) is recognized as a key player in the pathophysiology of PCOS, since IR and hyperinsulinemia directly and

Abbreviations: BAT, brown adipose tissue; CL, corpus luteum; CPT1α, Carnitine palmitoyltransferase1α; DHEA, dehydroepiandrosterone; IO2, Type II iodothyronine deiodinase; FSH, follicle stimulating hormone; GTT, Glucose Tolerance Test; ITT, Insulin Tolerance Test; IR, Insulin resistance; LH, luteinizing hormone; MCAD, medium-chain acyl-coenzyme A (CoA) dehydrogenasemedium-chain acyl-coenzyme A (CoA) dehydrogenase; OXPHO, Oxidative phosphorylation; P450C17, steroid 17 alpha-hydroxylase/17,20 lyase; 3β-HSD, 3β-Hydroxysteroid dehydrogenase; 17β-HSD, 17β-Hydroxysteroid dehydrogenase; STAR, Steroidogenic Acute Regulatory Protein; PGC1, PPARG Coactivator 1; PCOS, Polycystic ovary syndrome; PET-CT, Positron emission tomography–computed tomography; PPARα, Peroxisome proliferator-activated receptor α; T2D, Type 2 diabetes; UCP1, uncoupling protein 1.

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indirectly stimulate ovarian theca cells to secrete androgens and ultimately result in the augmentation of androgen excess [9–11]. It is estimated that about 85% of women with PCOS suffered from insulin resistance [12]. Therefore, we aim to explore and develop an effective option for the treatment of PCOS by improving insulin sensitivity. Increasing evidences show that the function and morphology of adipose tissue is aberrant in PCOS patients, and this abnormality is closely linked to insulin resistance even in the absence of obesity [11,13,14].

In addition to white adipose tissue (WAT), humans and small mammals possess brown adipose tissue (BAT) [15,16] that contributes to whole body metabolic homeostasis not only by uncoupling protein 1 (UCP1)-mediated thermogenesis but also by secretory cytokines such as adiponectin and FGF21. The amount of metabolically active BAT is inversely correlated with body mass index (BMI) and adiposity in adult human [17]. BAT activation is able to be a potential therapeutic option for the treatment of metabolic diseases such as PCOS. Recently, we and the other group demonstrated that BAT transplantation reversed obesity and insulin resistance in obese mice [18–20]. In addition, we showed that BAT transplantation in PCOS rat significantly increased insulin sensitivity and eventually ameliorated hyperandrogenism, acyclicity and infertility [21]. Interestingly, these beneficial effects of BAT transplantation are, at least in part, mediated by up-regulation of systemic adiponectin expression [21]. Since BAT transplantation is, however, unsuitable for clinical application, activating endogenous BAT could be an alternative novel therapeutic strategy for the treatment of PCOS in clinic.

In our recent study, we showed that rutin, a flavonoid, enhanced BAT activity and induced beige adipocytes formation in WAT, contributing to ameliorate obesity and insulin resistance in obese mice [22]. Therefore, we hypothesized that rutin could ameliorate PCOS by enhancing BAT activity. In the current study, we show that rutin treatment significantly activates endogenous deactivated BAT and ameliorates PCOS phenotypes including hyperandrogenism, acyclicity and infertility. Taken together, these results highlight that BAT deactivation is associated with the development of PCOS and BAT activation using rutin could be a useful therapeutic option for the treatment of PCOS in clinic.

2. Materials and methods

2.1. Animal

Female and male Sprague–Dawley rats (3 weeks old) were obtained from Vital River Laboratory Animal Technology Co Ltd. Five rats per cage were housed under constant environmental conditions in the Office of Laboratory Animal Welfare-certified animal facility with a 12-h light–dark cycle. Water and food were provided *ad libitum*. All animal studies were conducted with the approval of the Institutional Animal Care and Use Committee office Institute of Zoology, Chinese Academy of Sciences.

2.2. Induction of PCOS

DHEA used for establishing the PCOS model was purchased from Yangzhou Pharmaceutical Co., Ltd. (cat. no.H10940064). Female rats (4 weeks old) were subcutaneously injected with DHEA (6 mg/100-g body weight) dissolved in 0.2 ml of PBS for 20 consecutive days. Control rats (4 weeks old) were subcutaneously injected with 0.2-ml PBS for 20 consecutive days. The selection of successful PCOS development was based on the assessment of estrous cycle by vaginal cytology for 8 consecutive days.

2.3. Rutin treatment

Female rats in rutin-treated group were treated with rutin (100-mg/kg body weight) by gavage once a day for 3 weeks [23,24], and female rats in control group were treated with vehicle (DMSO, 5-μl/kg body weight) by gavage once a day for 3 weeks.

2.4. Assessment of estrous cycle

Based on the daily observation of leukocytes, cornified epithelial cells and nucleated epithelial cells from vaginal epithelial cell smears under light microscope, the stage of estrous cycle was determined. Mostly nucleated and some cornified epithelial cells were observed at proestrous stage; mostly cornified epithelial cells were present at estrous stage; both cornified epithelial cells and leukocytes were present at metestrus stage, and primarily leukocytes were visible at diestrus stage [25].

2.5. Glucose tolerance test and insulin tolerance test

For glucose tolerance tests (GTTs), female rats were fasted for 16 h (17:00–9:00) with free access to drink water and intraperitoneally injected with D-glucose (2.0-g/kg body weight). Blood glucose level was measured before and 15, 30, 60, 90 and 120 min after D-glucose injection. Glucose was measured with an Accu-Chek glucose monitor (Roche Diagnostics Corp). For the insulin tolerance test (ITT), female rates were fasted for 4 h (09:00–13:00) with free access to drink water and intraperitoneally injected with insulin (1-U/kg body weight) (Humulin; Eli Lilly). Blood glucose levels were measured before and 15, 30 and 60 min after insulin injection.

2.6. Resting metabolic rate

Oxygen consumption was measured by TSE lab master system as described previously [26]. The female rats were placed on the cages for 20–24 h, and then, VO_2 and VCO_2 were measured during the next 24 h. Female rats were maintained at 24 °C under 12-h light/dark cycle with free access to food and water during measurement.

2.7. Infrared thermography and core temperature

Rats (one rat per cage) were placed in cold chamber (4 °C) for up to 4 h with free access to food and water. Images were taken using an infrared digital thermographic camera (E60: Compact Infrared Thermal Imaging Camera; FLIR) and analyzed by FLIR Quick Report software (FLIR ResearchIR Max 3.4; FLIR). Core temperature was measured using a rectal probe connected with digital thermometer (Yellow Spring Instruments).

2.8. Micro PET/CT

PET/CT imaging was achieved with the Siemens Inveon Dedicated PET (dPET) System and Inveon Multimodality (MM) System (CT/SPECT) (Siemens Preclinical Solutions, Knoxville, TN, USA) at the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences. The female rats were allowed to fast overnight and were lightly anesthetized with isoflurane followed by a tail vein injection of ^{18}F–FDG (500 mCi). The female rats were subjected to PET/CT analysis at 60 min after radiotracer injection. Inveon Acquisition Workplace (IAW) software was used for scanning process. A 10-min CT X-ray for attenuation correction was scanned with a power of 80 Kv and an exposure time of 1100 ms before PET scan. Ten-minute static PET scans were acquired, and images were reconstructed by an OSEM3D algorithm followed by Maximization/Maximum *a Posteriori* (MAP) or FastMAP provided by IAW. The three-dimensional regions of interest (ROIs) were drawn over the guided CT images, and the tracer uptake was measured using the software of Inveon Research Workplace (IRW) (Siemens). Individual quantification of the ^{18}F–FDG uptake in each of the ROIs was calculated. The data for the accumulation of ^{18}F–FDG on micro PET images were expressed as the standard uptake values (SUV), which were determined by dividing the relevant ROI concentration by the ratio of the injected activity to the body weight. The data are presented as the mean ± S.E.M.

2.9. Fertility assessment

Female rats mated with proven stud males. Successful mating was determined by the occurrence of a vaginal plug. On 10 days after identification of vaginal plug, female rats were sacrificed to examine implantation sites to confirm pregnancy.

2.10. Blood analysis

Blood samples were collected from cardiac puncture under Avertin anesthesia. The plasma samples were stored at −80 °C until further analysis. Rat plasma levels of LH, FSH, NGF, FGF21, insulin (NanJing Jian Cheng Bioengineering Institute, China) and adiponectin (R&D Systems, USA) were analyzed using ELISA kits under manufacture's instruction. T3, T4, Estradiol, Progesteron, Testosteron, CHO, TG, HDL and LDL were analyzed from the Clinical Lab of 'The 306TH Hospital of PLA' (Chinese People's Liberation Army).

2.11. H&E staining and immunohistochemistry

Tissue were fixed in 4% paraformaldehyde overnight at room temperature and then embedded in paraffin. Sections with 5-μm thickness were stained with hematoxylin and eosin (H&E), then images were taken by microscope (DS-RI1; Nikon). The number of corpora lutea was counted based on morphology. Tissue specimens were blocked with 10% normal goat serum for 60 min, incubated with anti-UCP1 (1:400 dilution; Santa Cruz Biotechnologies) antibody overnight at 4 °C and then incubated with HRP-conjugated goat anti-rabbit IgG at room temperature for 1 h.

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