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Role of oxidative stress and low-grade inflammation in letrozole-induced polycystic ovary syndrome in the rat

Vivek Pandey [a], Anusha Singh [b], Ajit Singh [b], Amitabh Krishna [b], Uma Pandey [c], Yamini Bhusan Tripathi [a,*]

[a] Department of Medicinal Chemistry, Faculty of Ayurveda, Banaras Hindu University, Varanasi, India
[b] Department of Zoology, Faculty of Science, Banaras Hindu University, Varanasi, India
[c] Department of Gynaecology, Banaras Hindu University, Varanasi, India

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ABSTRACT

The aims of the current study were to examine the effects of temporal changes in oxidative stress (OS) and low-grade inflammation in letrozole-treated rats and to correlate these changes with the development of polycystic ovary syndrome (PCOS)-like features. Rats were treated with letrozole for 7, 15 and 21 days to induce PCOS. On day 7 of the treatment, a significant increase in serum testosterone and high sensitive C-reactive protein (hsCRP), the low-grade inflammatory marker, was found in the letrozole treated rats compared to control rats. Moreover, a decreased immunoexpression of insulin receptor coincided with increased body weight. The strong correlation between the levels of hsCRP and lipid peroxidation (LPO) suggests simultaneous development of low-grade inflammation and OS in response to hyperandrogenism, and the role of OS in a formation of cystic follicles in the letrozole animal PCOS model. Therefore, the results of the present study suggest that OS and low-grade inflammation (hsCRP) are the major causes of PCOS induction in this model.

1. Introduction

The polycystic ovary syndrome (PCOS) is the most common cause of infertility in women of reproductive age. It is a multifactorial disorder showing major features such as hyperandrogenism, hyperinsulinemia, obesity, insulin resistance, anovulation, and cystic follicles in the ovary [1]. If PCOS is not corrected it may lead to other serious consequences such as type II diabetes mellitus (DM), ovarian cancer or cardiovascular disorders [2]. Therefore, it is important to understand the exact etiology behind this disorder. The mechanism by which

* *Corresponding author at*: Department of Medicinal Chemistry, IMS, BHU, 221005, India. Tel.: +91 9415694450.
E-mail address: Yamini30@gmail.com (Y.B. Tripathi).
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obesity causes the PCOS-like changes in the ovary has not yet been fully understood. Obesity in PCOS is associated with insulin resistance and hyperinsulinemia [3]. Recent studies also showed that obesity is associated with a low-grade inflammation [4]. Insulin resistance (IR) and compensatory hyperinsulinemia seem to exert a direct stimulatory effect on low-grade inflammation in PCOS. Low-grade inflammation is associated with an increase in plasma levels of high sensitive C-reactive protein (hsCRP). Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are other biological markers of inflammation, which also increase in low-grade inflammation [4–6]. It has been shown earlier that obese persons with high level of hsCRP showed a greater risk of developing type II DM diabetes mellitus [7]. These findings suggest that markers of inflammation may be responsible for long-term consequences of PCOS. However, the mechanism by which low-grade inflammation induces pathogenesis of PCOS needs further investigation.

The oxidative stress (OS), which increases during inflammation, has also been reported as a potential cause of PCOS [8]. Increased OS has been shown to correlate with IR. In earlier studies, IR and hyperglycemia have been shown to increase OS, although in one study increased OS have also been demonstrated in non-obese PCOS without IR [9]. Some earlier studies have shown increased lipid peroxidation (LPO) in hyperglycemia [10]. An increase in reactive oxygen species (ROS) generation resulting from hyperglycemia has been observed in women with PCOS.

Obesity has also been shown to elevate OS, which in turn contributes to IR [11]. Studies done so far have suggested various ROS and antioxidants as biomarkers for PCOS patients [12]. However, the involvement of OS in the pathogenesis of PCOS is not yet fully substantiated. Various laboratory and domestic animal models have been developed to induce PCOS-like features. Mainly cystic follicles, hyperandrogenism and anovulation have been observed after treating adult females with various hormones, such as estradiol valerate, dehydroepiandrosterone, anti-progesterone, insulin, or human chorionic gonadotropin (hCG), or the neonates with testosterone [13]. However, the precise mechanism by which insulin resistance, oxidative stress and low-grade inflammation are developed in PCOS has not yet been investigated. Recently, a rat model for PCOS has been developed using non-steroidal aromatase inhibitor, letrozole [8,14]. In the present study, we examined temporal changes in morphology, oxidant-antioxidant status, serum hormone levels, and serum levels of low-grade inflammatory markers in letrozole-treated rats in order to explore the mechanism of PCOS pathogenesis.

2. Material and methods

2.1. Animal and experimental design

The protocols of animal experiments were approved by the Institutional Animal Ethical Committee (no. – Dean/12-13/CAEC/15). The rats utilized in this study were obtained from the central animal facility of Institute of Medical Sciences of Banaras Hindu University, India. All animals were housed under controlled temperature (24 ± 2 °C) with a relative humidity of 40–55% and 12 h of light and dark cycle with unlimited access to water and dry pelleted rat food. Twenty-four adult female albino rats (10 weeks old) of CF strain weighing 75–80 g with two consecutive estrous cycles were randomly divided into 4 groups. Each group comprised six rats. Groups 2, 3 and 4 were treated with letrozole (Sun pharmaceuticals, Mumbai, India) for 7, 15 and 21 days, respectively. Group 1 (control) rats received the vehicle (1% aqueous solution of carboxymethyl cellulose – 2 mL/kg of body weight (bw) once daily fed orally Rats of groups 2–4 received letrozole (in 1% aqueous solution of carboxymethyl cellulose solution) at a concentration of 3 mg/kg fed orally once daily. The dose was selected based on a preliminary experiment in which rats were treated with different doses of letrozole ranging from 1 to 5 mg/kg bw. Significant changes were observed in rats treated with a minimum dose of 3 mg/kg bw, so this dose was selected for further study. Whereas the control group was euthanized after 21 days, the letrozole-treated rats were euthanized after 7, 15 or 21 days after the treatment. The estrous cycles of each rat were monitored through daily examination of vaginal cytology. The body weight of each rat was recorded on every third day during the entire course of the experiment. Twenty-four hours after the last treatment, animals were euthanized and serum was kept at −20 °C for hsCRP and testosterone assay. One ovary of each rat was fixed in Bouin's fluid for histological examination, whereas the other ovary was stored at −40 °C until used for biochemical and immunoblot analysis of superoxide dismutase (SOD), catalase and lipid peroxidation (LPO).

2.2. Histomorphometry of ovarian follicles

The ovaries fixed in Bouin's fluid were processed for histological examination. Thin serial paraffin sections (5 μm) of the ovary were stained with hematoxylin-eosin. Follicular diameter, thickness of the granulosa and theca layers and follicular changes were examined *via* microscopy and imaging software. Different types (healthy antral and cystic) of follicles were counted in every fifth serial sections of each ovary. The numbers of healthy antral and cystic follicles in the ovaries collected after 7, 15 and 21 days of the letrozole treatment (corresponding to different stages off PCOS development) were compared with those of the control animals. Follicles showing features such as hypertrophied granulosa cells, pyknotic cell nuclei in the granulosa cells and an abnormal oocyte were categorized as abnormal follicles. Histological changes were categorized and scored according to the severity of changes in the ovary: no changes = 0; mild = +; moderate = ++; and intense = +++ (Table 2).

2.3. CRP and testosterone determination

Serum hsCRP, a marker of low-grade inflammation, was determined by ELISA kit (Diagnostics Biochem, Dorchester, ON, Canada) [15]. Testosterone, an endocrine marker of PCOS, was quantitatively determined by Chemiluminescent Microparticle immunoassay (Architect Testosterone Reagent kit, Abbott, Dublin, Ireland) [16].

2.4. Activity of antioxidant enzymes and lipid peroxidation

Total (Cu–Zn and Mn) superoxide dismutase activity was determined as described earlier [17] with slight modification