Theriogenology xxx (2014) 1–10

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Cellular and molecular mechanisms of pentoxifylline's beneficial effects in experimental polycystic ovary

Mohammad A. Rezvanfar [a,b], Sepideh Saadat [c], Habib A. Shojaei Saadi [d], Parisa Mansoori [c], Sarah Saeedi [c], Maziar Gooshe [e], Maryam Baeeri [b], Mohammad Abdollahi [a,b,*]

[a] Division of Toxicology, Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[b] Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Pharmaceutical Sciences Branch, Islamic Azad University, Iran
[d] Centre de Recherche en Biologie de la Reproduction, Université Laval, Québec City, Québec, Canada
[e] School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 24 November 2014
Accepted 26 November 2014

Keywords:
Polycystic ovary syndrome
Oxidative stress
Hyperandrogenism
Pentoxifylline
TNF-α

ABSTRACT

Chronic low-grade inflammation and oxidative stress (OS) appear to be two main pathways involved in the pathogenesis of polycystic ovary (PCO) syndrome. Therefore, targeting these pathways by means of anticytokine and antioxidant agents might be a therapeutic alternative approach to the current treatments of PCO syndrome. In this study, we investigated the protective effects of pentoxifylline (PTX), a drug with antioxidant and anti–tumor necrosis factor alpha (TNF-α) properties, in hyperandrogenism-induced PCO rats. The inflammatory and OS responses and their connections with ovarian functionality in induced PCO rats were investigated through ovarian histopathologic examination and a series of biochemical measurements including serum estradiol, progesterone, testosterone, insulin, and TNF-α, ovarian and serum lipid peroxidation, total antioxidant power, and reactive oxygen species. Experimental PCO was induced in rats by oral administration of letrozole (1 mg/kg body weight) for 21 consecutive days. In a different group, PTX was administrated orally (50 mg/kg/d) for 21 days simultaneous with letrozole to assess its potential protective effects. The letrozole-induced PCOs were characterized by irregular cycles, high incidence of subcapsular ovarian cysts with diminished or scant granulosa cell layers, increased number of atretic preantral and antral follicles, and absence of CL. In addition, the letrozole-induced PCO rats exhibited notable increase in lipid peroxidation and reactive oxygen species of serum and ovary, serum testosterone, insulin, and TNF-α and significant decline in total antioxidant power, serum estradiol, and serum progesterone. Our results indicated that all the identified pathologic parameters and biochemical characteristics in letrozole-induced PCO rats in this study were preserved close to normal levels by simultaneous PTX treatments. Present results demonstrate that there is a direct connection between ovarian dysfunction and increased OS and inflammation in PCO. For the first time, the beneficial effects of PTX as a powerful antioxidant and TNF-α blocker in hyperandrogenism-induced PCO are reported.

1. Introduction

It is estimated that 40% to 50% of cases of infertilities or subfertilities in couples are caused by female reproductive disorders [1]. Polycystic ovarian syndrome (PCOS) as a

* Corresponding author. Tel./fax: +98 21 66959104.
E-mail address: mohammad.abdollahi@utoronto.ca (M. Abdollahi).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.11.034

lifetime complex multigenic endocrinopathy is the leading cause of anovulatory infertility in women of reproductive age [2]. It is characterized by increasing androgen secretion, menstrual irregularity, oligo-ovulation or anovulation, polycystic ovaries (PCOs), infertility, and pregnancy complications. In addition to reproductive features, PCOS is associated with several metabolic disorders such as type 2 diabetes and cardiovascular diseases including abdominal obesity, insulin resistance, hyperinsulinemia, glucose intolerance, hypertension, and metabolic syndrome [3].

The precise mechanisms and etiology of PCOS are unknown. Although, PCOS is considered as a hormone-dependent disorder, recent evidence proposed the substantial role of oxidative stress (OS) and chronic low-grade inflammation in the pathogenesis of PCOS and its metabolic associations [1,4,5]. We have previously shown the role of oxidative and nitrosative stress and inflammatory responses (overproduction of cytokines) in the pathogenesis of hyperandrogenism-induced rat PCO [6,7]. Higher concentration of the serum tumor necrosis factor alpha (TNF-α) in PCO and the crosstalk between OS and proinflammatory cytokines particularly TNF-α seem to play a pivotal role in the progression of ovarian cystogenesis, follicular atresia, and ovarian dysfunction [8,9]. Moreover, follicular atresia and diminishing quantity and quality of oocytes during ovarian cystogenesis have been found to increase OS in PCO [4,10]. Hyperandrogenemia is seen in the preliminary phases of most PCOS patients, which impairs maturation of developing follicles in the ovaries and consequently develops cystogenesis [11]. Currently, the goal of treatment for PCO(S) patients is to control hyperandrogenism, restoration of reproductive cyclicity, and initiation of ovulation. In this respect, the most conventional therapy of PCOS is metformin, which helps to induce ovulation [12]. In spite of prolonged effective application of conventional treatments for PCOS, adverse effects such as cardiovascular complications and multiple pregnancies [13] are their drawbacks. Therefore, none of the current therapies can fully improve all the clinical features of the PCOS and studies are still being conducted to find better drugs [14].

Because chronic low-grade inflammatory state and OS are thought to be the two main pathways involved in the pathogenesis of PCOS [1,4,8,10], targeting these pathways by means of anticytokine and antioxidant agents seems an alternative approach to current PCOS treatments.

Pentoxifylline (PTX) as a methylxanthine derivative is much in demand as a cardiovascular drug, which has been widely used to ameliorate peripheral vascular diseases. Pentoxifylline enhances flexibility of blood cells and flow [15] and acts as a vasodilator because of nonselective inhibition of phosphodiesterase (PDE) leading to augmented generation of cyclic nucleotides such as cAMP and cyclic guanosine monophosphate. It has been proven to be a drug with preventive effects of overproduction of toxic-free radicals [16], an inhibitor of xanthine oxidase, an enzyme involved in the generation of oxygen-free radicals [17–19], and potent inhibitor of cytokines, particularly TNF-α, which is a key component in inducing the generation of reactive oxygen species (ROS) from mitochondria [18,20]. Because of anti–TNF-α effect, growing evidence has demonstrated that PTX has broad-spectrum beneficial effects to suppress or modulate the progression of OS-related disorders such as diabetes [18], colitis [20], varicocele-associated infertility [21], and acute pancreatitis [5].

To the best of our knowledge, this is the first study designed to evaluate the effects of a PDE inhibitor with anti–TNF-α effects in PCO. We hypothesized that treatment with PTX as dual inhibitors of TNF-α production and xanthine oxides can contribute to a simultaneous blockade of OS and proinflammatory condition in the ovaries of the hyperandrogenism-induced PCO in murine model, keeping local and systemic inflammatory response and maintaining normal folliculogenesis.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Gmbh, Munich). Pentoxifylline from Amin Pharmaceutical Co. (Tehran, Iran), letrozole from Soha Pharmaceutical Co. (Tehran, Iran), rat TNF-α ELISA kit from Bender MED Systems (Vienna, Austria), ethyl acetate from Fluka (Tehran, Iran), Krebs-ringer-bicarbonate, steroid hormone radioimmunoassay kits from Neogen (Tehran, Iran) were used in this survey.

2.2. Animals

Forty adult female albino *Wistar* rats (200 ± 10 g) with normal estrous cycle were used in this study. All animals had *ad libitum* access to pelleted food and tap water and housed under controlled temperature (22 °C–25 °C) with a relative humidity of 40% to 55% and 12 hours lights and dark cycle. Only females with at least three consecutive 4 to 5 days regular estrous cycles were included in this experiment. Vaginal smear was taken daily to determine the stage of the estrous cycle during the entire treatment up to the autopsy day and injections were started on the same day of the estrous cycle in all rats.

Rats were randomly separated into four groups (10 rats per group) including control, letrozole-induced PCO, PTX-treated non-PCO, and PTX-treated PCO groups. The experiments were performed in accordance with the National Health guidelines, and the study protocol was approved by the institutional review board and ethics committee of Tehran University of Medical Sciences with code number 91-02-33-16968.

2.3. Treatments

The control group received only vehicle (0.9% NaCl solution) orally, one time daily. The PCO group was gavaged with letrozole once daily at the concentration of 1 mg/kg orally dissolved in 0.9% NaCl as described previously [6,7]. The rats in PTX-treated non-PCO group received 50 mg/kg of PTX orally and the rats in PTX-treated PCO group were given letrozole orally (1 mg/kg dissolved in 0.9% NaCl), 30 minutes after PTX treatment (50 mg/kg, orally). The treatment period was 21 days and the effective doses of PTX and letrozole were selected according to our pilot studies and the previous experiments [6,7,16].