Original Research Article

Effect of Thiazolidinedione Amide on Insulin Resistance, C-reactive Protein and Endothelial Function in Young Women with Polycystic Ovary Syndrome

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Abstract

Purpose: To investigate the effect of thiazolidinedione amide (TZDA) treatment on high-sensitivity C-reactive protein (hsCRP) levels and endothelial dysfunction in patients with polycystic ovary syndrome (PCOS).

Methods: Twenty five women (mean age 24.7 ± 3.9 years; mean body mass index (BMI), 23.2 ± 4.0 kg/m^2) with PCOS were treated with 15 µM TZDA daily for 12 months. Serum levels of testosterone, leutenizing hormone (LH), follicle stimulating hormone (FSH), sex hormone-binding globulin (SHBG), insulin and hsCRP were measured. BMI, hirsutism scores and insulin sensitivity indices were also calculated prior to and after TZDA treatment. Brachial artery responses to stimuli was used to measure arterial endothelium and smooth muscle function prior to and after the treatment.

Results: TZDA treatment caused a significant ($p < 0.05$) decrease in serum testosterone from 93.1 ± 40.3 to 54.8 ± 19.5 ng/dl and fasting insulin concentration from 11.9 ± 6.8 to 9.23 ± 5.13 U/mL. Insulin resistance index significantly ($p < 0.05$) improved and hirsutism score decreased significantly from 11.6 ± 2.0 to 6.8 ± 2.0. BMI, waist circumference, serum total cholesterol, low-density lipoprotein (LDL)-cholesterol, FSH and LH levels remained almost unchanged. Twenty-four of the women reverted to regular menstrual cycles. SHBG levels showed a significant ($p < 0.05$) increase from 24.8 ± 9.5 to 49.1 ± 13.5 nmol/L after TZDA treatment. Serum hsCRP levels decreased ($p < 0.05$) from 0.25 ± 0.1 to 0.09 ± 0.02 mg/dL while endothelium-dependent vascular responses significantly improved ($p < 0.05$) following TZDA treatment (9.9 ± 3.9 vs 16.4 ± 5.1%).

Conclusion: TZDA treatment improves insulin sensitivity, decreases androgen production and improves endothelial dysfunction in young women with PCOS.

Keywords: Thiazolidinedione amide, Insulin sensitivity, Endothelial dysfunction, Polycystic ovary syndrome

INTRODUCTION

Anovulation and hyper-androgenism are the characteristic features of polycystic ovary syndrome (PCOS). There are about 6 % of the women of reproductive age who suffer from PCOS [1]. The common features of the PCOS are resistance to insulin accompanied by simultaneous hyper-insulinemia [2,3]. The hyper-insulinemia induces enhanced ovarian androgen

production and reduces serum sex hormone–binding globulin concentration [4-6]. Inhibition of insulin secretion by diazoxide [7], metformin [8] or diet [9,10] in polycystic ovary syndrome patients leads to reduction in serum free testosterone concentrations. Insulin resistance in adolescent girls with hyperandrogenism clearly indicates involvement of hyper-insulinemia polycystic ovary syndrome [11].

It is reported that PCOS women exhibit significantly adverse coronary heart disease (CHD) risk profiles at relatively young age, suggesting that these increases may result in premature coronary atherosclerosis [12-15].Long incubation period in CHD associated with metabolic abnormalities observed in the teens or twenties among PCOS women might translate to measurable vascular abnormalities by middle age [14]. The atherosclerotic disease is characterised by the endothelial cell dysfunction which is an early event in the process of lesion formation [16-18]. Assessment of endothelial function by measuring flow-mediated dilatation (FMD) of the brachial artery is currently regarded as a potential tool for predicting CHD [19].

Dysfunction of the endothelium is associated with insulin resistance resulting in increased risk of atherosclerosis in insulin-resistant subjects, such as those with PCOS [20-22]. Atherosclerosis, a chronic inflammatory process and the measure of inflammatory markers like C-reactive protein (CRP) provide a method for assessment of cardiovascular risk [23]. Studies have demonstrated a correlation between insulin resistance and CRP concentrations [24-26]. Taking into consideration the concept that reducing insulin resistance may prevent early atherosclerosis, insulin sensitizers have been used in women with PCOS [26].

Troglitazone, amember of the thiazolidinedione (TZD) family, was found to have beneficial effects on insulin sensitivity and ovarian function in women with PCOS [27-29]. In a previous study, endothelial dysfunction in women with PCOS was improved by troglitazone therapy [30]. However, troglitazone was taken off the market as a result of concerns over its hepatotoxicity. The aim of our present study is to evaluate the efficiency of thiazolidinedione amide (TZDA) (Figure 1) therapy on insulin resistance, serum concentrations of pro-inflammatory markers and endothelial dysfunction in young women with PCOS.
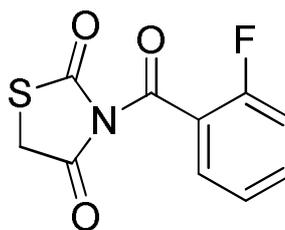

Figure 1: Structure of thiazolidinedione amide (TZDA)

EXPERIMENTAL

Ethical statement

The study was approved and performed according to the guidelines of the Ethics Committee for Human Studies of Shandong University, Jinan, Shandong, China and assigned it the reference number SU-023/2014. A written consent was obtained from all the patients after explanation of the nature, purpose and potential risks of the study.

Patients

Twenty five women (mean age 24.7 ± 3.9 years) with PCOS were enrolled in our study. The selection of the patients was made on the basis of presence of two of the following three features: oligo- or anovulation; clinical Ferriman-Gallwey score>8 and/or biochemical signs of hyperandrogenism and polycystic ovaries. Biochemical criteria include abnormal luteinizing (LH):follicle-stimulating hormone (FSH) ratio (> 2) and/or enhanced levels of testosterone. The PCO was diagnosed, on the basis ultrasound showing presence of 12 or more follicles per ovary 2-9 mm in diameter, and/or increased ovarian volume (> 10 ml). The mean cycle length in the patients was 52.7 ± 12.8 days and had normal thyroid, renal and hepatic functions. Women with pregnancy, use of oral contraceptives in 6 months period before the study, anti-androgens, anti-diabetics and known cardiovascular disease (CVD) were excluded from the study. For exclusion of Cushing's syndrome and late-onset congenital adrenal hyperplasia dexamethazone suppression test and follicular phase serum 17-OH progesterone determination were done.

Clinical examination and laboratory evaluation

After 3 days of the treatment blood samples were collected, an oral glucose tolerance test and trans-vaginal ultrasonography were performed. Chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA) was used to measure

serum concentrations of FSH, LH, testosterone, prolactin and sex hormone-binding globulin (SHBG). Glucokinase technique was used to measure serum glucose by the treatment of blood serum with glucokinase. The lipid profile measurement of the levels of total cholesterol, high- and low-density lipoprotein (HDL and LDL)-cholesterol and triglyceride was also performed using commercial enzymatic methods (Aeroset automated analyzer, Abbott Diagnostics, IL, USA). Friedewald's formula was used to calculate LDL-cholesterol. Chemiluminescent enzyme immunoassay (Immulite 1000 Analyser) and chemiluminescent enzyme immunoassay (Immulite 2000) were used for measuring plasma insulin levels and plasma hsCRP concentrations respectively. To determine insulin resistance various methods like fasting insulin, the homeostasis model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI) were used. Insulin resistance was estimated using HOMA score.

Arterial endothelium and smooth muscle functions were measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli employing ultrasonography. A 7.5 MHz transducer (Toshiba Power Vision 8000) was used to capture B-mode ultrasound images to determine brachial artery diameter. The scans were video-recorded later on analysis. We measured the arterial diameters during rest, during reactive hyperemia (FMD), again at rest and after treatment with 0.4 mg sublingual NTG. The end-diastolic arterial diameter was measured from one to the other media-adventitia interface and the measurements were made in triplicate at baseline, every 20 s after reactive hyperemia and after administration of NTG. All the women were treated with 15 µM TZDA per day. The patients were examined every month for 12 months and were admitted to the clinical research center. Clinical examinations and laboratory evaluations were performed, the results obtained were then analysed and compared with baseline values.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 11.5) and the results are expressed as mean ± SD. Characteristics of distribution were tested using Kolmogorov–Smirn of test. Spearman's rank correlation was used for these variables. Differences between means were analyzed using Student's unpaired t-test and considered statistically significant at $p < 0.05$.

RESULTS

In our study all the 25 women enrolled completed the treatment without any report of adverse effects at the dosage of 15 µM TZDA per day for 12 months. The results showed no enhancement in serum transaminases in all the tested patients during the TZDA treatment period. TZDA treatment for 12 months caused twenty-three patients to revert to the regular ovulatory cycles. The length of the cycles at the end of the treatment was 31.3 ± 4.5 days. All the clinical characteristics and hormonal parameters for the patients were recorded (Table 1).

The results from our study revealed that TZDA treatment for 12 months induces only minor change in body weight without affecting waist circumferences. There was no significant change in total cholesterol, LDL, LH and FSH levels. TZDA treatment lead to a significant decrease in the hirsutism score from a baseline value of 11.6 ± 2.0 to 6.8 ± 2.0 after 12 months. Serum triglyceride concentrations were decreased and serum HDL concentrations were increased after treatment but these changes were statistically insignificant ($p > 0.05$).

Table 1: Patient characteristics and hormone studies before and after TZDA treatment

Variable	Before TZDA (n = 25)	Post-TZDA (n = 25)	*P*-value
BMI (kg/m2)	23.20 ± 4.00	27.10 ± 4.40	0.41
Waist circumference (cm)	78.45 ± 7.54	82.35 ± 7.80	0.54
Ferriman–Gallwey score	11.60 ± 2.10	8.30 ± 2.20	0.10
LH (IU/l)	8.00 ± 5.60	8.30 ± 6.90	0.48
FSH (IU/l)	3.98 ± 3.34	6.00 ± 3.10	0.49
Testosterone (ng/dl)	93.10 ± 40.30	54.80 ± 19.50	0.03
SHBG (nmol/l)	24.80 ± 9.50	49.10 ± 13.50	0.03
Total cholesterol (mg/dl)	165.50 ± 40.40	159.90 ± 40.00	0.45
Triglyceride (mg/dl)	125.00 ± 98.79	113.50 ± 69.60	0.12
HDL-cholesterol (mg/dl)	45.70 ± 9.89	51.00 ± 10.78	0.10
LDL-cholesterol (mg/dl)	93.25 ± 19.90	101.30 ± 30.80	0.28

Values expressed as means ± S.E.

Serum testosterone levels decreased significantly after TZDA treatment from 93.1 ± 40.3 to 54.8 ± 19.5 ng/dl whereas SHBG levels increased from 24.8 ± 9.5 to 49.1 ± 13.5 nmol/l. Serum hsCRP level also decreased significantly after 12 months of treatment from 0.25 ± 0.1 to 0.09 ± 0.02 mg/dL ($p < 0.05$).

Examination of the endothelium-dependent (FMD) vascular responses after TZDA treatment showed a significant improvement with an increase in value from 8.78 ± 4.8 to 17.2 ± 4.79 % after 12 months (Figure 3). There was no change in baseline artery diameter and endothelium in-dependent vascular responses after TZDA treatment (Table 3).

DISCUSSION

The endothelium releases different autocrine and paracrine substances which play vital roles in vascular homeostasis. In normal state endothelium suppresses pro-atherogenic processes, like monocyte and platelet adhesion, oxidation of LDLs, synthesis of inflammatory cytokines, smooth muscle proliferation and migration, and platelet aggregation. Atherosclerotic disease is characterized by onset of endothelial dysfunction [16,17]. For the detection of preclinical cardiovascular disease endothelial function is assessed using different methods like brachial artery ultrasound. Disorders like hypertension, hypercholesterolemia and insulin resistance are accompanied by reduced endothelium-dependent vasodilatation [16]. It is reported that secondary hyperinsulinemia is the key factor responsible for the hyperandrogenism characteristic of PCOS, which is attributed to increased stimulation of the activity of the cytochrome P450c 17a in the ovary.

There is a decrease in the concentration of circulating SHBG and increase in concentrations of free androgens in the blood in hyperinsulinemia [22].

Table 2: Glucose metabolism in patients before and after TZDA therapy (mean ± SEM, n = 25)

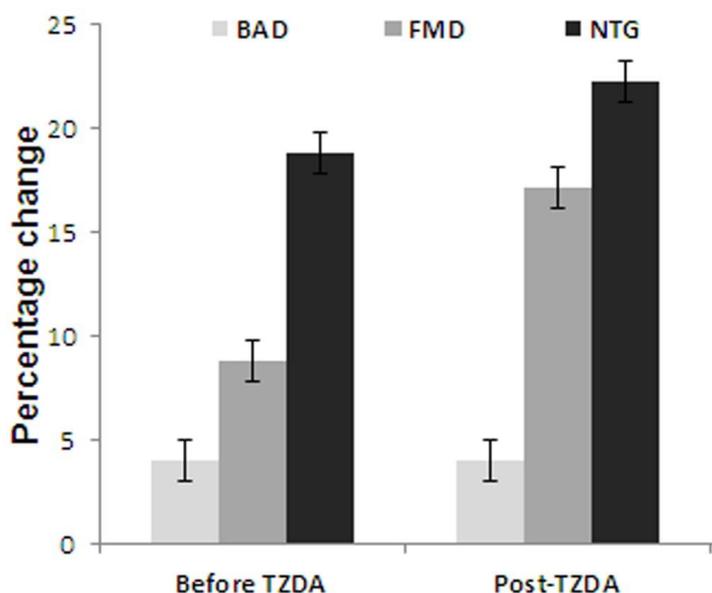| Variable	Before TZDA (n = 25)	Post-TZDA (n = 25)	*P*-value
Fasting glucose (mg/dl)	89.61 ± 8.50	88.7 0± 8.78	0.31
Fasting insulin (mU/ml)	11.90 ± 6.80	9.23 ± 5.13	0.02
HOMA	3.21 ± 1.80	2.34 ± 0.67	0.02
QUICKI	0.29 ± 0.03	0.41 ± 0.06	0.03

Figure 3: Brachial artery responses in patients with PCOS after TZDA treatment.

Table 3: Brachial artery responses, expressed as percentage dilatation from baseline (mean ± SEM, n = 25)

Variable	Before TZDA	Post-TZDA	*P*-value
Baseline artery diameter (BAD) (mm)	4.02 ± 0.38	4.04 ± 0.41	0.786
FMD (%)	8.78 ± 4.80	17.20 ± 4.79	0.032
NTG (%)	18.81 ± 4.91	22.30 ± 5.97	0.295

Both lean and obese women with PCOS have peripheral insulin resistance and hyperinsulinemia that appear to play a pathogenic role in the disease [22]. Previous studies have shown an endothelial dysfunction in women with PCOS [22]. Accumulating evidence also suggests that atherosclerosis represents a chronic inflammatory process and inflammatory markers like CRP provide an adjunctive method for global assessment of cardiovascular risk [22-26]. Recent data also suggest that CRP may directly promote endothelial dysfunction by increasing the synthesis of soluble adhesion molecules, increasing monocyte chemo-attractant protein secretion and facilitating macrophage LDL uptake [25]. In previous studies, high hsCRP levels and a link between insulin resistance and elevated CRP levels were shown in women with PCOS [22].

The use of insulin-sensitizing agents in the ovarian abnormalities of PCOS patients indicated the involvement of insulin. Such strategies also resulted in beneficial effects on the levels of markers for cardiovascular disease. Endothelial dysfunction and low-grade chronic inflammation being at the initial stages of atherosclerosis attracted us to investigate the effect of TZDA on hs CRP levels and endothelial dysfunction in women with PCOS.

Rosiglitazone treatment was shown to improve ovulatory function and decrease insulin resistance in women with PCOS. The results from our study clearly demonstrated a significant decrease in insulin resistance and androgen levels along with improvement of ovulatory cycles in women with PCOS after TZDA treatment for 12 months. Earlier it was reported that rosiglitazone improves endothelial dysfunction and decrease hsCRP levels in non-diabetic patients with coronary artery disease and also in non-diabetic patients with metabolic syndrome. The results from our study reveal that TZDA treatment improves endothelium-dependent vasodilatation and decreases serum levels of pro-inflammatory marker hsCRP levels in PCOS patients. TZDA treatment also improved endothelial dysfunction and hsCRP levels in non-obese young women with PCOS.

CONCLUSION

Thus TZDA treatment improves insulin sensitivity, helps to restore ovulation and decrease androgen production in women with PCOS. Therefore, TZDA can be used for the treatment of PCOS.

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