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Troglitazone Improves Ovulation and Hirsutism in the Polycystic Ovary Syndrome: A Multicenter, Double Blind, Placebo-Controlled Trial*

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ABSTRACT

We hypothesized that the administration of troglitazone, an insulin-sensitizing agent of the thiazolidinedione class, would improve the ovulatory dysfunction, hirsutism, hyperandrogenemia, and hyperinsulinemia of polycystic ovary syndrome (PCOS) patients. Four hundred and ten premenopausal women with PCOS in a multicenter, double blind trial were randomly assigned to 44 weeks of treatment with placebo (PBO) or troglitazone [150 mg/day (TGZ-150), 300 mg/day (TGZ-300), or 600 mg/day (TGZ-600)]. We compared changes in ovulatory function (by monitoring the urinary level of pregnanediol-3-glucuronide daily), hirsutism (by a modified Ferriman-Gallwey scoring method), hormonal levels (total and free testosterone, androstenedione, sex hormone-binding globulin, LH, FSH, and the LH/FSH ratio), and measures of glycemic parameters (fasting levels of glucose, insulin, hemoglobin A_{1c}, and the glucose and insulin areas under the curve during an oral glucose challenge) among study groups.

Of the 410 patients recruited, 305 (74.4%) met evaluability criteria and were included in the analyses. The patients' baseline characteristics were similar across all treatment arms. Ovulatory rates were significantly greater for patients receiving TGZ-300 and TGZ-600 than for those receiving PBO (0.42 and 0.58 *vs.* 0.32; $P < 0.05$ and 0.0001, respectively). Of PCOS patients treated with TGZ-600, 57% ovulated over 50% of the time compared with 12% of placebo-treated patients. There was a significant decrease in the Ferriman-Gallwey score with TGZ-600 compared with PBO (0.22 ± 0.53 *vs.* -2.21 ± 0.49; $P < 0.05$, respectively). Free testosterone decreased and sex hormone-binding globulin increased in a dose-related fashion with troglitazone treatment, and all three troglitazone treatment groups were significantly different from placebo. Nearly all glycemic parameters showed dose-related decreases with troglitazone treatment. The total number and severity of adverse events (including elevations in liver enzymes) and the proportion of patients withdrawn from the study due to the development of adverse effects were similar between treatment groups.

Troglitazone improves the ovulatory dysfunction, hirsutism, hyperandrogenemia, and insulin resistance of PCOS in a dose-related fashion, with a minimum of adverse effects. (*J Clin Endocrinol Metab* **86:** 1626–1632, 2001)

THE POLYCYSTIC OVARY syndrome (PCOS) is a heterogeneous condition of unknown etiology characterized by oligoovulation and androgen excess (1). This disorder affects approximately 4% of reproductive aged women (2) and is one of the most common causes of oligoovulatory infertility (3). Women with PCOS often demonstrate insulin resistance, which results in compensatory hyperinsulinemia (4–6). Hyperinsulinemia, in turn, leads to increased blood levels of androgens because of the effects of insulin in lowering sex hormone-binding globulin (SHBG) production (7, 8) and increasing ovarian androgen secretion (9, 10).

Existing therapies for PCOS have focused on suppressing androgen production or effect or inducing ovulation. More recently, and consistent with the premise that insulin resistance is an important etiological cause of PCOS, several studies have demonstrated a beneficial effect of insulin-lowering agents in this disorder (11–24). However, reports examining the effect of these drugs on ovulatory function in PCOS have generally been small (~30 subjects), brief (<3 months), and have not included dosing studies. In addition, the effects of these drugs on other PCOS-related androgenic features, such as hirsutism, has yet to be determined.

Troglitazone is an insulin-sensitizing agent of the thiazolidinedione class, with a postinsulin receptor mechanism of action. In preliminary studies troglitazone has demonstrated

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a dose-related decrease in circulating insulin and androgen levels in obese PCOS patients (12, 14). We hypothesized that the administration of troglitazone would demonstrate a dose-related improvement in ovulatory dysfunction and hirsutism of PCOS. To test our hypothesis we conducted a 44-week multicenter, double blind, placebo-controlled trial of PCOS patients using three different doses of troglitazone.

Subjects and Methods

Subjects

Of the 782 premenopausal women with suspected PCOS who were screened for this study, 410 patients qualified and were randomized to double blind treatment. Of these, 305 (74.4%) patients completed the study sufficiently to meet evaluability criteria. For the purposes of this study, PCOS was diagnosed by 1) the presence of chronic ovulatory dysfunction, defined as intermenstrual intervals of 45 days or more or a total of eight or fewer menses per yr; 2) hyperandrogenemia, defined as a serum level of free testosterone (T) greater than the upper normal limit used in the central laboratory for this study (*i.e.* ≥21.8 pmol/L); and 3) the exclusion of other disorders, such as nonclassic adrenal hyperplasia (25), thyroid dysfunction, and hyperprolactinemia. These diagnostic (*i.e.* inclusion) criteria for PCOS are consistent with the suggestions arising from a preliminary consensus conference sponsored by the NICHHD, NIH, in April 1990 (1).

Other exclusionary criteria included unresolved medical conditions; hysterectomy and/or oophorectomy, type 1 or type 2 diabetes mellitus, significant cardiovascular disease, active cancer within the past 5 yr, and participation in another investigational study within the past 30 days. The use of medications known or suspected to affect reproductive or metabolic functions within 60 days of study entry was prohibited. This study was approved by, and conducted according to, the guidelines of the institutional review boards of each of the participating centers. All subjects provided written informed consent.

Study protocol

After a 2-week baseline evaluation, eligible patients were randomized in a double blind fashion to one of the following treatment groups: placebo (PBO), or troglitazone [150 mg/day (TGZ-150), 300 mg/day (TGZ-300), or 600 mg/day (TGZ-600)]. Parke-Davis Pharmaceutical Research, Inc. (the sponsor) provided the medication. Patients were asked to follow a weight maintenance diet throughout the study to minimize the effect of weight changes on the disease state.

Patients returned for assessment 4 weeks after the start of the double blind, randomized phase and every 8 weeks thereafter (weeks 4–44). At each visit vital signs and body measures were obtained and recorded, and blood and urine specimens were obtained to monitor safety (see below). Laboratory efficacy parameters were assessed, and an oral glucose tolerance test (OGTT) was performed at weeks 0, 20 and study completion. For the OGTT, blood samples were obtained at −10, 0, 30, 60, 90, and 120 min after the oral ingestion of 75 g glucose.

Patients collected daily morning (first void) urine samples in specially designated containers. The samples were frozen and transported to the study site at the appropriate clinic visit. In addition, patients were instructed to record in a daily diary menstrual bleeding and spotting and urine collection information.

The presence and extent of hirsutism were determined at the randomization visit (week 0), week 20, and at study completion using a modification of the Ferriman-Gallwey (F-G) scoring method (26). Patients in the study were requested not to use electrolysis, waxing, or plucking for removal of unwanted hair, except for treatment of lower legs and forearms.

Safety assessment

Safety evaluation included vital signs, blood tests (liver function tests, chemistry panel, complete blood counts), and urinalysis. Initially these were evaluated at entry into the protocol, after 4 weeks, and every 8 weeks thereafter. However, after approximately half of the patients were recruited, the sponsor issued new safety and prescribing guidelines for the use of troglitazone, and all patients remaining underwent additional study visits to ensure that liver function tests were monitored every 2 weeks during the first 6 months of the double blind phase and monthly thereafter until the end of the trial. The presence of a large ovarian cyst (>30 mm) mandated periodic transvaginal ultrasound evaluations until resolution. In addition, measurements of endometrial thickness were performed by transvaginal ultrasound. If the single wall thickness was more than 14 mm an endometrial biopsy was performed to exclude endometrial hyperplasia or carcinoma, if deemed appropriate by the investigator.

Patients, either during the initial screening or throughout the study, who demonstrated elevations in the hepatic enzymes aspartate aminotransferase (AST) or alanine aminotransferase (ALT) of 1.5 times or more than the upper normal limits were retested within 7 days. If the ALT or AST level rose to greater than 3 times the upper normal limit, the patient was immediately withdrawn from the study and followed periodically until her laboratory values returned either to normal or to prestudy levels.

All nonsterilized patients were asked to use a barrier method of contraception (condom or diaphragm) during intercourse. Those patients conceiving during the study were immediately withdrawn from the study, and periodic follow-up information was obtained.

Laboratory analysis

Blood samples were analyzed for total and free T, androstenedione (A^4), SHBG, FSH, LH, PRL, estradiol, TSH, and 17-hydroxyprogesterone (17-HP). The safety profile consisted of blood samples analyzed for electrolytes, glucose, blood urea nitrogen, creatinine, uric acid, total protein, albumin, liver function tests (AST, ALT, lactate dehydrogenase, and total, direct and indirect bilirubin), lipids (total, low density lipoprotein, and high density lipoprotein cholesterol, and triglycerides), complete hematological profile, serum pregnancy test, and urinalysis. Blood samples for efficacy determination were analyzed for total and free T, A^4, SHBG, FSH, and LH. Glucose and insulin were measured at all time points during the OGTT.

The specifics of the laboratory methods are detailed as follows. Serum free T was determined using equilibrium dialysis against a buffer containing tritium-labeled T. Total serum T was measured by RIA after extraction with hexane-ethyl acetate and column chromatography. The binding capacity of SHBG was directly measured in serum using a displacement technique that uses ammonium sulfate precipitation of free and protein bound steroid in place of equilibrium dialysis or gel filtration. A^4 was measured by a direct RIA. These assays were performed by Endocrine Sciences, Inc. (Calabasas Hills, CA). All other analyses, including chemistry, hematology, lipids, and urinalysis, were performed by Medical Research Laboratories, Inc. (Highland Heights, KY). Glucose levels were measured by the hexokinase procedure (747–200, Hitachi, Hialeah, FL), hemoglobin A_{1c} (HbA_{1c}) levels by high performance liquid chromatography (Variant, Bio-Rad Laboratories, Inc., Richmond, CA), and insulin levels by RIA. Triglyceride levels were measured using the standard lipase, glycerokinase, glycerol-3-phosphate oxidase, and peroxidase method, and high density lipoprotein cholesterol was determined by the precipitation and enzymatic method of lipoprotein (Hitachi 747). Low density lipoprotein cholesterol levels were calculated using the Friedewald formula. Routine hematology and clinical chemistry analyses were performed by standard methodology using Coulter STKS and Hitachi 747 instrumentation. The level of pregnanediol-3-glucuronide was determined by an enzyme immunoassay competitive assay.

Test (efficacy) parameters

The efficacy of troglitazone at modifying the following parameters was compared between all four study groups.

Ovulation. Ovulatory frequency was assessed for each patient by monitoring the daily urinary levels of the progesterone metabolite pregnanediol-3-glucuronide, as described previously (27). The ovulation rate was calculated for each patient as the number of ovulatory events observed divided by the number of events that could have occurred during the time period studied (*i.e.* number of actual ovulatory events/number

of potential ovulatory events). The ovulation rates were averaged across patients within each treatment group.

Hirsutism. The change in hirsutism score was assessed only in those patients exhibiting an F-G score of 6 or more at baseline.

Androgen and gonadotropin levels. The mean changes from baseline in serum total and free T, A^4, SHBG, LH, FSH, and LH/FSH ratio were compared among groups.

Markers of glucose and insulin homeostasis. The mean change from baseline in the fasting levels of glucose, insulin and HbA_{1c}, and the glucose and insulin areas under the curve (AUC) during the OGTT were evaluated.

Statistical analysis

Primary treatment comparisons were those between each troglitazone treatment and placebo. Tests were two-sided and conducted at $\alpha = 0.05$. In general, the methods used for the analyses were analysis of covariance with treatment and center in the model and baseline as the covariate, for all parameters except incidence of ovulation. For ovulation, ANOVA with treatment and center in the model was performed. All *P* values reported from the analyses of covariance and ANOVAs are from stepdown trend tests via contrast statements. The *P* values reported for comparison of ovulation responder rates are from the Cochran-Mantel-Haenszel test of general association. The comparison of time to ovulation was performed using the log-rank test. Unless otherwise indicated values represent the mean ± SE.

Results

Baseline patient characteristics and patient participation

As noted, 305 (74%) patients met the evaluability criteria and were included in the analyses. The clinical characteristics of these patients are depicted in Table 1, and their hormonal and metabolic profiles are shown in Table 2. Overall, there were no significant differences in baseline features between treatment groups. The percentage of patients not completing the entire study and the reasons for termination were similar among all treatment groups (*i.e.* those completing 40 weeks of study were 40.8%, 42.3%, 34.3%, and 39.6% for the PBO, TGZ-150, TGZ-300, and TGZ-600 treatment groups, respectively). The principal reasons for patients not completing the study were early termination of the study by the sponsor (range, 11.5–19.8%) and lack of compliance (range, 5.0–13.6%). The percentage of patients withdrawing from the study due to adverse events ranged from 4–7%; this was not different between treatment arms.

Effect of troglitazone on ovulation in PCOS

As the onset of metabolic effects of troglitazone is gradual, we initially did not consider the first 12 weeks of therapy when calculating ovulatory rates. Hence, when analyzing ovulatory function we considered all evaluable patients, *i.e.* those completing at least 84 days (12 weeks) in the study. Approximately 85% of patients remained in the study long enough to be eligible for analysis of ovulation and menses data.

The ovulatory event rates observed from week 12 onward was significantly greater for patients receiving TGZ-300 and TGZ-600 than for those given PBO (Fig. 1). The dose-related positive effect of troglitazone on ovulation remained even

TABLE 1. Baseline characteristics of evaluable patients

Parameter	Placebo	Troglitazone		
		150 mg	300 mg	600 mg
No.	73	78	77	78
Race				
White (%)	78.1	74.4	83.1	83.1
Black (%)	12.3	17.9	7.8	9.1
Hispanic (%)	4.1	2.6	3.9	3.9
Other (%)	5.5	5.1	5.2	3.9
Age (yr)	30.1 ± 6.0	28.9 ± 5.4	29.2 ± 5.8	29.0 ± 5.2
Body mass index (kg/m^2)	37.9 ± 8.3	37.3 ± 8.3	35.3 ± 9.3	35.6 ± 8.3
Waist/hip ratio	0.89 ± 0.08	0.89 ± 0.08	0.86 ± 0.08	0.88 ± 0.09
Time from diagnosis (yr)	3.1 ± 4.4	4.1 ± 4.7	4.0 ± 4.3	3.4 ± 4.3
No. of cycles in past 12 months	4.5 ± 2.7	4.3 ± 3.1	4.4 ± 2.7	4.6 ± 3.0
Smoking (%)	20.5	20.5	20.8	23.4

Values are the mean ± SD unless indicated otherwise.

TABLE 2. Baseline hormonal and metabolic characteristics of evaluable patients

Parameter	Placebo	Troglitazone		
		150 mg	300 mg	600 mg
No.	73	78	77	78
Total testosterone (ng/mL)	0.57 ± 0.03	0.63 ± 0.04	0.64 ± 0.03	0.63 ± 0.03
Free testosterone (pg/mL)	10.62 ± 0.61	11.72 ± 0.73	11.55 ± 0.69	10.92 ± 0.56
Androstenedione (ng/mL)	1.89 ± 0.08	1.92 ± 0.08	2.09 ± 0.10	1.98 ± 0.07
Sex hormone-binding globulin (nmol/L)	36.14 ± 2.03	39.51 ± 2.56	41.39 ± 2.44	40.69 ± 2.18
LH (mIU/mL)	9.45 ± 0.67	9.83 ± 0.61	11.35 ± 0.97	10.85 ± 0.96
FSH (mIU/mL)	6.01 ± 0.22	6.03 ± 0.21	6.20 ± 0.22	6.64 ± 0.21
LH/FSH	1.67 ± 0.12	1.67 ± 0.09	1.92 ± 0.14	1.65 ± 0.10
Fasting insulin (μIU/mL)	23.6 ± 1.9	32.4 ± 5.8	23.8 ± 2.8	20.4 ± 1.7
Fasting glucose (mg/dL)	94.0 ± 1.2	96.6 ± 1.9	.92.4 ± 1.2	93.1 ± 1.1
Glycosylated hemoglobin A_{1c} (%)	5.30 ± 0.10	5.30 ± 0.10	5.00 ± 0.10	5.20 ± 0.10
Area under curve, glucose (mg/dL·h)	283.4 ± 6.5	288.4 ± 8.8	269.6 ± 8.0	275.3 ± 7.6
Area under curve, insulin (μIU/mL·h)	257.7 ± 18.2	264.0 ± 23.8	235.4 ± 21.7	230.4 ± 19.4

Values are the mean ± SE.

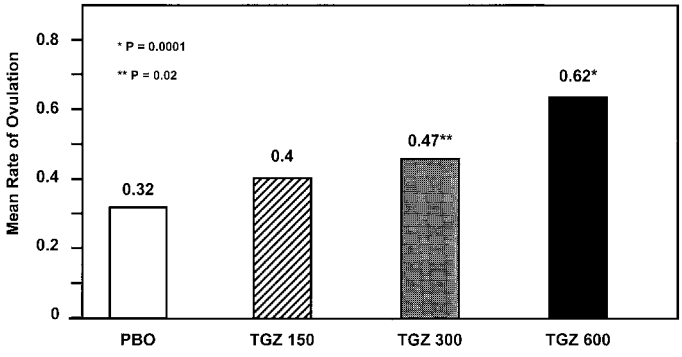

FIG. 1. The mean rate of ovulation in patients with PCOS increased in a dose-related fashion with troglitazone treatment and was significantly different from PBO for TGZ 300 and TGZ 600 groups, but not for TGZ 150 patients.

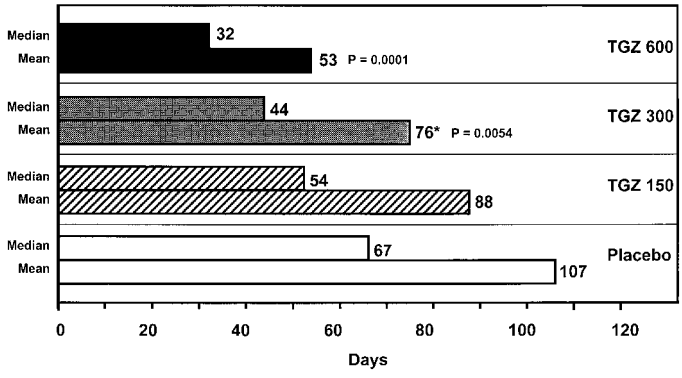

FIG. 2. Median time (days) to first ovulation in women with PCOS treated with TGZ 300 and TGZ 600 was significantly less than when patients were treated with PBO or TGZ 150.

when ovulatory events observed within the first 12 weeks of the study were included in the analysis (0.32 ± 0.04, 0.39 ± 0.04, 0.42 ± 0.04, and 0.58 ± 0.04 for PBO, TGZ-150, TGZ-300, and TGZ-600, respectively; $P < 0.05$ and $P < 0.0001$ for TGZ-300 and TGZ-600 *vs.* PBO, respectively). In all, 57% of PCOS patients treated with TGZ-600 ovulated over 50% of the time, respectively, compared with 12% of the PBO-treated patients. Finally, the time to first ovulation was significantly less for PCOS patients treated with TGZ-300 or TGZ-600 compared with women treated with either PBO or TGZ-150 (Fig. 2). The improvement in menstrual cycle regularity mirrored that of ovulation (data not shown).

To determine what factors might be predictive of ovulatory response to treatment we combined patients treated with TGZ-300 and TGZ-600 and subdivided them into tertiles according to ovulatory rates. Those patients in the bottom tertile (ovulatory rate, <33%) were compared with those in the highest tertile (ovulatory rate, >67%). Patients with a higher ovulatory rate during the study were slightly older (30.2 ± 0.8 *vs.* 26.1 ± 0.9 yr, respectively; $P < 0.003$), less obese (33.3 ± 1.0 *vs.* 38.7 ± 2.1 kg/m^2, respectively; $P < 0.02$), had more episodes of vaginal bleeding in the year before entering the study (5.3 ± 0.5 *vs.* 3.3 ± 0.6, respectively; $P < 0.02$), had lower basal insulin (14.4 ± 1.1 *vs.* 27.0 ± 4.2 μIU/mL; $P < 0.009$), and had lower free T (9.8 ± 0.7 *vs.* 12.9 ± 1.3 pg/mL, respectively; $P < 0.04$) and higher SHBG levels (43.8 ± 2.9 nmol/L *vs.* 31.7 ± 5.0 nmol/L, respectively, $P < 0.04$), than patients with low ovulatory rates. The degree of response to treatment for any of the parameters studied was not different between patients with low and high ovulation rates.

In-study pregnancies

Fourteen patients were withdrawn from the study due to pregnancy: 2 in the PBO-treated and 12 in the troglitazone-treated arms. Two additional patients were found to be pregnant at their last visit: 1 a PBO-treated patient and the other a patient who was taking TGZ-300. Thus, there were a total of 3 pregnancies on PBO and 13 on troglitazone during the study. Overall, the number of unexpected pregnancies among patients treated with either TGZ-300 or TGZ-600 was greater than that among women treated with either TGZ-150 or PBO (5.9% *vs.* 1.4%, respectively; $P < 0.02$).

Effect of troglitazone on hirsutism in PCOS

Approximately 50% of randomized patients had a baseline F-G score of 6 or more and at least one follow-up observation and were thus evaluable for analysis of the hirsutism scores. At baseline, there was no difference in the number of hirsute patients (n = 57, 56, 55, or 62) or in the mean basal F-G scores (14.23 ± 0.94, 14.77 ± 0.84, 14.27 ± 0.95, and 14.42 ± 0.84) among the PBO, TGZ-150, TGZ-300, or TGZ-600 treatment groups, respectively. Overall, there appeared to be a dose-related decrease in F-G scores with troglitazone treatment (0.22 ± 0.53, −0.51 ± 0.53, −0.80 ± 0.53, and −2.21 ± 0.49 for PBO, or TGZ-150, TGZ-300, or TGZ-600, respectively), a difference that became significant from PBO at the TGZ-600 dose ($P < 0.0003$; Fig. 3). The effect of therapy on excess hair growth was not evaluated in patients whose initial F-G score was less than 6.

Effect of troglitazone on androgen and gonadotropin levels in PCOS

For these analyses all patients who had a baseline and at least one follow-up observation were considered evaluable. Free T decreased in a dose-related fashion with troglitazone treatment, and all three troglitazone treatment groups were significantly different from PBO (Table 3). Consistent with this finding, SHBG levels increased in a dose-related fashion,

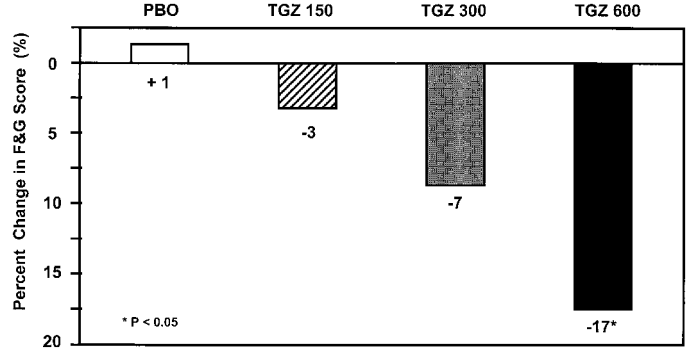

FIG. 3. The percent decrease in hirsutism, measured by a modified F-G score, in patients treated with PBO, TGZ 150, TGZ 300, and TGZ 600. Note that the decrease in hirsutism score was significantly different from placebo with the use of TGZ 600.

TABLE 3. Mean change from baseline in androgens levels, gonadotropin levels, and markers of insulin resistance in PCOS patients after treatment with troglitazone

Parameter	Placebo	Troglitazone		
		150 mg	300 mg	600 mg
No.	73	78	77	78
Total testosterone (ng/mL)	−0.04 ± 0.03	−0.06 ± 0.03	0.01 ± 0.03	−0.01 ± 0.03
Free testosterone (pg/mL)	−1.11 ± 0.57	−2.72 ± 0.55[a]	−3.07 ± 0.55[b]	−4.13 ± 0.55[b]
Androstenedione (ng/mL)	−0.01 ± 0.06	−0.16 ± 0.06	−0.11 ± 0.06	−0.14 ± 0.06
Sex hormone-binding globulin (nmol/L)	2.22 ± 2.57	8.05 ± 2.48	18.69 ± 2.49[b]	29.12 ± 2.49[b]
LH (mIU/mL)	−0.56 ± 1.01	0.43 ± 0.97	−1.11 ± 0.98	−0.18 ± 0.96
FSH (mIU/mL)	−0.10 ± 0.27	0.32 ± 0.26	−0.51 ± 0.26	0.55 ± 0.26
LH/FSH	−0.05 ± 0.13	−0.02 ± 0.12	−0.10 ± 0.12	−0.14 ± 0.12
Fasting insulin (μIU/mL)	−5.0 ± 1.7	−5.8 ± 1.7	−9.8 ± 1.8[a]	−10.8 ± 1.7[a]
Fasting glucose (mg/dL)	−1.7 ± 1.2	−1.8 ± 1.2	−4.0 ± 1.2	−5.3 ± 1.2[a]
Glycosylated hemoglobin A_{1c} (%)	0.01 ± 0.03	−0.07 ± 0.03	−0.07 ± 0.03	−0.14 ± 0.03[a]
Area under curve, glucose (mg/dL·h)	−13.8 ± 5.0	−21.1 ± 4.9	−30.5 ± 5.0[a]	−33.5 ± 4.9[a]
Area under curve, insulin (μIU/mL·h)	−23.8 ± 10.8	−70.9 ± 10.8[a]	−78.4 ± 11.0[b]	−103.4 ± 10.8[b]

Values are the mean change from baseline, adjusted for baseline and center bias, ±SE.
[a] Significantly different from placebo, $P = 0.050–0.001$.
[b] Significantly different from placebo, $P < 0.001$.

and the changes observed in the TGZ-300 and TGZ-600 treatment groups were significantly different from PBO. Although total T and A^4 levels appeared to decrease with therapy, this difference did not reach significance. Finally, neither basal gonadotropin levels nor the LH/FSH ratio changed with therapy.

Effect of troglitazone on glycemic parameters in PCOS

For this analysis all patients who had a baseline and at least one follow-up observation (*i.e.* intent to treat) were considered. Because the first postrandomization efficacy laboratory measurements were not taken until week 20, about 70% of the randomized patients are included in these analyses. Nearly all glycemic parameters showed dose-related decreases with troglitazone treatment (Table 3). TGZ-600 reduced circulating insulin by 53%, fasting glucose by 5.7%, HbA_{1c} by 2.7%, the AUC for glucose by 12.2%, and the AUC for insulin by 44.9%. Patients receiving troglitazone had small increases in body weight (−0.77, +0.51, +0.78, and +1.01 kg for PBO, TGZ-150, TGZ-300, and TGZ-600, respectively.) Only TGZ-300 and TGZ-600 were statistically significant ($P = 0.03$ and 0.02, respectively).

Adverse events

Overall, there was no significant difference between treatment groups in the proportion of all or only associated adverse events, and none of the patients died during the study. The proportion of patients withdrawn from the study due to the development of potentially associated adverse effects was not different among treatment groups (1.9%, 2.9%, 2.9%, and 5.9% for PBO, TGZ-150, TGZ-300, and TGZ-600, respectively). There was no difference in the proportion of patients treated with PBO, or TGZ-150, TGZ-300, and TGZ-600 who experienced either a mild (1.5–3 times the upper normal limit) or a severe (>3 times the upper normal limit) increase in ALT (5.2%, 7.1%, 6.1%, and 3.0% and 2.0%, 1.0%, 4.0%, and 3.0%, respectively) or AST (5.2%, 6.2%, 5.1%, and 4.1% and 2.1%, 0%, 2.0%, and 1.0%, respectively). Across treatment groups (PBO, TGZ-150, TGZ-300, and TGZ-600) 1.0%, 1.9%, 2.9%, and 2.0% of patients, respectively, were withdrawn from the study due to the development of an abnormal liver function test. Of note, at screening 46 (5.8%) patients seen had elevations in serum ALT (n = 43) and/or AST (n = 23). Two (0.2% of the total screened) of these patients had an ALT value greater than 3 times the upper normal limit.

Discussion

Insulin resistance appears to be central to the pathogenesis of PCOS. The precise molecular basis for the insulin resistance has not been elucidated, but it appears that a postreceptor defect (6) results in profound hyperinsulinemia that is disproportionate to the degree of obesity. The excess insulin in PCOS appears to augment LH-stimulated androgen secretion from the ovary (9, 10) and to decrease circulating SHBG levels, resulting in higher levels of free androgens (7, 8). In the present controlled trial of over 300 patients we demonstrated that troglitazone improves ovulatory function and hirsutism in PCOS. We speculate that these clinical improvements result from attenuation of the associated insulin resistance, hyperinsulinemia, and hyperandrogenemia.

The present study clearly demonstrates the therapeutic effect of troglitazone on the ovulatory dysfunction of PCOS. About 60% of cycles were ovulatory among patients receiving TGZ-600, and 57% of these patients ovulated at least 50% of the time. This ovulatory rate is similar to that reported by other investigators for ovulation induction with clomiphene citrate in PCOS (28–30). Although TGZ-300 was moderately successful in stimulating ovulation, TGZ-150 was ineffectual in improving ovulatory function. The importance of including a placebo-treated control group is highlighted by the fact that 32% of untreated cycles in our PCOS patients were apparently ovulatory, consistent with the findings of other investigators (19, 31). Overall 75% of our placebo-treated patients ovulated in less than 50% of the cycles.

Few other investigators have examined the effect of insulin-lowering or insulin-sensitizing agents administered alone on ovulatory function. Ovulation rates of 28% and 43% were reported when troglitazone (400 mg/day) was administered for 4 and 12 weeks, respectively, in uncontrolled studies of small numbers (<20) of subjects (21, 22). Nestler and col-

leagues treated 61 obese PCOS patients with either metformin (1500 mg/day) or placebo for 35 days (17). These investigators noted that 34% of patients treated with metformin alone ovulated compared with 4% of controls, similar to the ovulatory response noted by other investigators in a smaller uncontrolled study (18). Nestler and colleagues also treated 44 women with PCOS with either placebo or the insulin sensitizer D-*chiro*-inositol (1200 mg/day) for 6–8 weeks and observed that 86% of treated patients ovulated (19). However, these latter studies primarily addressed the ovulatory response during 1 treatment cycle and not the restoration of ovulatory function over time.

As is observed with the use of clomiphene (28, 32–36), patients who were more affected (*i.e.* greater degree of obesity or higher free T or basal insulin levels) were also less likely to ovulate in response to troglitazone. Furthermore, there was no difference in the insulin response to an OGTT between patients with high and low ovulatory rates after TGZ-600 treatment, suggesting that this drug was effective in inducing ovulation in PCOS patients even in the absence of marked hyperinsulinemia. Similarly, Nestler and Jakubowicz reported that lean women with PCOS and with a relatively normal insulin response to an oral glucose challenge experienced a reduction in hyperandrogenism with the use of metformin (1500 mg/day) (37). Hence, troglitazone appears to be effective for improving ovulatory function in patients with PCOS regardless of the degree of clinically evident hyperinsulinemia. However, and as with other therapeutic agents, the degree of therapeutic response tends to be less the more severe the disorder.

Despite extensive contraceptive counseling, 16 patients became pregnant during the study: 3 in the placebo-treated and 13 in the troglitazone-treated arms. Although not an objective of the study, the number of pregnancies occurring was 4-fold higher among those patients treated with either TGZ-300 or TGZ-600 than in those given PBO or TGZ-150. There is no reason to believe that the proportion of patients not using adequate contraception would have been varied among the treatment groups. A number of other pregnancies have been reported with the use of troglitazone in hyperandrogenic patients (22, 38), although outcome data are not yet available. Troglitazone is categorized as a pregnancy class B drug, with no ill effects demonstrated in animal models. Nonetheless, additional studies documenting the impact of troglitazone on the fetus and pregnancy outcome are required.

Consistent with the improvement in androgen levels, we observed a dose-related decrease in hirsutism with troglitazone treatment. When treated with TGZ-600, hirsute PCOS patients experienced a 15% decrease in F-G score by 20 weeks of therapy. As most previous studies using insulin sensitizers in PCOS have been relatively short, it is not surprising that the impact of this therapy on hirsutism has not been systematically studied. In 2 studies in which this clinical feature was assessed, no difference in F-G score could be documented after the administration of metformin (1500 mg/day) for at least 4 months (15, 24). However, it is probable that these studies were of insufficient power to detect a difference, because they included less than 25 patients each, and not all were hirsute.

Various investigators have observed improvements in hyperinsulinemia and hyperandrogenemia when treating small numbers of PCOS patients with up to 400 mg/day troglitazone (12, 14, 21, 39). In the present study we observed a clear dose response, with TGZ-600 providing the greatest degree of improvement in insulin and androgen levels. TGZ-600 reduced circulating insulin by 53% and the insulin response to an oral glucose challenge by 45%. Similarly, this dose of drug reduced mean circulating free T levels by 38%, primarily due to a 71% rise in mean SHBG levels. Nonetheless, it is interesting to note that the mean free T levels after TGZ therapy remained above the upper normal limit of our laboratory (21.8 pmol/L).

We observed a mean increase in body weight of about 1 kg for the highest dose of TGZ used that represents only a 1% change in body weight for this population. Hence, the overall improvement in PCOS symptoms cannot be explained by an indirect action of the drug on body weight.

Rare, but serious, acute hepatic failure associated with troglitazone use in patients with type 2 diabetes has been reported (40–43). The majority appears to be related to idiosyncratic drug-related hepatocellular damage, and there does not appear to be any association with gender, age, daily dose, or the use of concomitant medication (42). Although the overall frequency of this adverse event is rare, strict prescribing guidelines and frequent monitoring of liver function tests have been instituted for type 2 diabetes. Recently, the manufacturer has withdrawn the marketing of troglitazone for type 2 diabetes. In our population of over 300 reproductive-aged PCOS patients the administration of troglitazone, regardless of dose, was not associated with an increased frequency (±2%) or withdrawals (±2%) due to liver function abnormalities.

Treatment of PCOS patients with insulin-sensitizing agents has various potential advantages over traditional therapies, including 1) correcting both the metabolic and the endocrinological aberrations of the disorder; 2) permitting the resumption of normal endogenous ovulatory function, with little or no risk of ovarian hyperstimulation and multiple gestation; and 3) possibly decreasing their long-term risk of type 2 diabetes mellitus (44–46). We conclude that troglitazone, primarily in a dose of 600 mg/day, improves the ovulatory dysfunction, hirsutism, hyperandrogenemia, and hyperinsulinemia of patients with PCOS, with a minimum of adverse effects.

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