Changes in Insulin Sensitivity in Response to Troglitazone Do Not Differ Between Subjects With and Without the Common, Functional Pro12Ala Peroxisome Proliferator-Activated Receptor-γ2 Gene Variant:

Results from the Troglitazone in Prevention of Diabetes (TRIPOD) study

Soren Snitker, MD,PHD[1], **Richard M. Watanabe, PHD**[2], **Ifeanyi Ani, BA**[1], **Anny H. Xiang, PHD**[2], **Aura Marroquin, RN, BSN**[2], **Cesar Ochoa, MD**[2], **Jose Goico, MD**[2], **Alan R. Shuldiner, MD**[1], and **Thomas A. Buchanan, MD**[2]

[1]University of Maryland, Baltimore School of Medicine, Baltimore, Maryland [2]Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

OBJECTIVE—We have tested whether the Pro12Ala variant of the peroxisome proliferator-activated receptor (PPAR)-γ nuclear receptor involved in thiazolidinedione (TZD) action accounted for the failure of troglitazone to increase insulin sensitivity in nondiabetic Hispanic women with previous gestational diabetes treated in the Troglitazone in Prevention of Diabetes (TRIPOD) study.

RESEARCH DESIGN AND METHODS—Ninety-three women assigned to troglita-zone had intravenous glucose tolerance tests at randomization and after 3 months of treatment with troglitazone, 400 mg/day, and were genotyped for the Pro12Ala variant of the PPAR-γ gene. Subjects were divided into tertiles based on their change in minimal model insulin sensitivity (S_i) during the first 3 months of troglitazone treatment.

RESULTS—The mean changes in S_i in the bottom, middle, and top tertiles of S_i response were -0.21 ± 0.57, 0.91 ± 0.26, and 2.58 ± 1.32 min^{-1} per μU/ml · 10^{-4}, respectively. Frequencies of the Ala/− genotype were 30, 22, and 26% in the same three tertiles ($P = 0.77$). Analysis of phenotypes by genotype revealed only small differences between the Pro/Pro and Ala/− groups, respectively, in baseline S_i (2.76 ± 0.19 vs. $2.33 \pm 0.33 \times 10^{-4}$ min^{-1} per μU/ml; $P = 0.27$), the change in S_i after 3 months of troglitazone treatment (1.19 ± 0.17 vs. 0.93 ± 0.30; $P = 0.46$), and the cumulative incidence of diabetes during a median follow-up of 30 months (13 vs. 17%; $P = 0.66$).

CONCLUSIONS—Among young Hispanic women at high risk for type 2 diabetes, the Pro12Ala variant of the PPAR-γ receptor gene did not explain the failure of ~1/3 of subjects to increase their insulin sensitivity when placed on troglitazone at a dose of 400 mg/day.

Thiazolidinedione (TZD) drugs have a variety of biological actions that are affected mainly through binding to peroxisome proliferator-activated receptor (PPAR)-γ nuclear receptors.

Address correspondence and reprint requests to Soren Snitker, MD, PhD, Department of Medicine, Division of Endocrinology, Diabetes, and Nutrition, University of Maryland, Baltimore School of Medicine, 660 W. Redwood St., Howard Hall Rm. 598-B, Baltimore, MD 21201-1596. ssnitker@medicine.umaryland.edu..
S.S. and R.M.W. contributed equally to this work.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

These receptors are expressed at high levels in fat tissue (1) and are of demonstrated importance for fat cell differentiation and whole-body insulin sensitivity. We have described (2) a common genetic variant in the PPAR-γ2 isoform, resulting in a proline-to-alanine substitution in position 12 of the receptor protein. This variant makes a prime candidate as a possible pharmacogenetic determinant of TZD response. First, the disparate physical properties of proline and alanine residues suggest that this polymorphism has functional consequences. Second, in clinical studies, Pro12Ala genotype is a determinant of insulin sensitivity and susceptibility to obesity and diabetes (3-8). Third, in vitro studies by Deeb et al. (9) and Masugi et al. (10) show reduced binding of the Ala12 protein to the PPAR-responsive element of several genes and decreased transactivation in the presence of increasing concentrations of a TZD. Lastly, heterozygosity for the Ala12 allele is frequent enough that it might be a common cause of TZD responsiveness (8).

Several reports indicate that the clinical response to TZDs varies. Some of the variation in glycemic responses could be due to the inclusion of patients with very low endogenous insulin levels. Insulin sensitization is not expected to lower glucose levels to an important degree in such patients. However, studies in which direct measurements of insulin sensitivity have been made reveal that some individuals do not experience an increase in insulin sensitivity when they are exposed to a TZD (11,12). In the Troglitazone in Pre- vention of Diabetes (TRIPOD) study (12), nondiabetic women with recent gestational diabetes whose change in insulin sensitivity during the first 3 months of troglitazone treatment was in the lowest tertile for treated subjects had a mean change in S_i that wasslightly less than zero and similar to placebo-treated subjects. Clinical and metabolic characteristics at baseline and compliance with study medications were not useful in distinguishing these "nonresponders" from women whose insulin sensitivity increased in response to the drug (12). For the present report, we genotyped women in the troglitazone arm of the TRIPOD study to determine whether the Ala variant at the 12 position of the PPAR-γ receptor gene accounted for the lack of response to the insulin-sensitizing effects of troglitazone. As a secondary focus, we tested the effects of the Ala variant on several physiologic variables and on the risk of diabetes during troglitazone treatment.

RESEARCH DESIGN AND METHODS

The rationale, methods, and results from TRIPOD have previously been presented (12,13). Briefly, subjects were recruited from Los Angeles County Women's and Children's Hospi- tal. Pivotal eligibility criteria were Hispanic ethnicity, previous gestational diabetes, and a sum of five oral glucose tolerance test plasma glucose concentrations ≥625 mg/dl (34.7 mmol/l), predicting a 70% risk of diabetes in the next 5 years (14). Women were randomized to placebo or troglitazone (400 mg/day), which was administered in a double- blind fashion. Enrollment continued until 266 subjects were randomized. The study protocol included a tolbutamide-modified, frequently sampled intravenous glucose tolerance test (15) performed at baseline and repeated after 3 months of treatment. The subjects were maintained on the drug for a median of 30 months after randomization and tested for diabetes with fasting glucose levels every 3 months and 75-g oral glucose tolerance tests (16) annually.

Genotyping

Women who completed the baseline and 3-month intravenous glucose tolerance tests were genotyped for the Pro12Ala polymorphism by the PCR restriction-fragment-length polymorphism technique. In addition to 93 subjects randomized to troglitazone, genotypes were available on 55 subjects randomized to placebo. The DNA was spun down in a 96-well plate and amplified with standard reaction and cycling conditions in 50-μl reactions containing PCR buffer, 10 mmol dNTPs, 12.5 pmol sense primer (5′-GCCAATTCAAGCCCAGTC-3′), 12.5 pmol antisense primer (5′-GATAT GTTTGCAGACAGTGTATCAGTGAAG

GAATCGCTTTCCG-3′), and 1 unit of *Taq* polymerase (Gibco, Carlsbad, CA). An aliquot of the amplified DNA was subjected to electrophoresis through a 3% agarose gel, stained with ethidium bromide, and DNA visualized by ultraviolet transillumination to confirm the presence of the predicted 270-bp product. Digestion was performed for 12 h at 60°C with the restriction enzyme BstU1. The digested samples were then subjected to electrophoresis through a 3% agarose gel, stained with ethidium bromide, and DNA visualized by ultraviolet transillumination. DNA product sizes for the PPAR-γ2 genotypes were: Pro/Pro, 270; Pro/Ala, 270/227/43; and Ala/Ala, 227/43 bp.

Analysis

The post hoc power calculation was performed with the Power and Precision Release 2.00 package (Biostat, Englewood, NJ). Insulin sensitivity (S_i) was calculated using the Minmod program (15) from glucose and insulin values originating from the frequently sampled intravenous glucose tolerance test. Nonresponders were defined as women in the bottom tertile of change in S_i (12). The acute insulin response to glucose (AIR_g) was calculated with the trapezoidal method as the incremental area under the insulin curve during the first 10 min after the glucose injection. The disposition index, a measure of β-cell compensation for insulin resistance, was calculated as the product of S_i and AIR_g (15). Statistical analyses were performed with SAS software (version 8.2; SAS, Cary, NC). Allele frequency differences were compared by χ^2. Comparison of continuous traits among groups was performed by multiple regression (GLM procedure), adjusting for age and, where appropriate, BMI. Variables were transformed to approximate a univariate normality where appropriate. Unbiased group estimates were calculated as least squares means as part of the GLM procedure. Values of $P < 0.05$ were considered statistically significant. Summary statistics are reported as unadjusted means ± SD.

RESULTS

There were 69 Pro/Pro, 23 Ala/Pro, and 1 Ala/Ala troglitazone-treated subject, yielding genotype frequencies of 0.87 (Pro) and 0.13 (Ala). Genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium. The 23 Ala/Pro and 1 Ala/Ala subjects were collapsed into one Ala/− group to facilitate statistical analysis.

The Pro/Pro and Ala/− groups did not differ significantly in terms of age (Pro/Pro, 35 ± 7, and Ala/−, 34 ± 7 years; $P = 0.29$), BMI (Pro/Pro, 31 ± 6, and Ala/−, 30 ± 5 kg/m^2; $P = 0.88$), or fasting glucose at entry (Pro/Pro, 95 ± 11, and Ala/− 93 ± 13 mg/dl; $P = 0.54$). Minmod S_i also did not differ significantly between the two groups at entry (Pro/Pro, 2.76 ± 0.19, and Ala/−, 2.33 ± 0.33 min^{-1} per μU/ml · 10^{-4}; $P = 0.27$).

Response tertiles

Subjects were divided into response tertiles based on their change in S_i after 3 months of treatment. The mean changes in S_i in the bottom, middle, and top response tertiles were −0.21 ± 0.57, 0.91 ± 0.26, and 2.58 ± 1.32 min^{-1} per μU/ml · 10^{-4}, respectively. Among the three tertiles, there was no trend for baseline S_i, which averaged 2.84 ± 1.78, 2.05 ± 1.23, and 3.02 ± 2.50 min^{-1} per μU/ml · 10^{-4} in the bottom, middle, and top response tertiles, respectively. A response tertile × genotype χ^2 test did not deviate significantly from the values expected in the absence of a genotype effect ($P = 0.77$) (Table 1). Of note, the response of the single Ala/Ala individual was in the middle tertile, with a δ value of 0.87 min^{-1} per μU/ml · 10^{-4}.

Development of diabetes

During the subsequent period, during which subjects were maintained on troglitazone (median duration 30 months), the cumulative incidence of diabetes among those treated with

troglitazone did not differ significantly by genotype and was reduced to a similar extent compared with placebo in the two genotypes (Fig. 1).

Continuous variables

The post hoc power calculation showed that we had 80% power to detect effects on continuous variables >0.67 times the SD of the measured variable. S_i increased to a similar extent in the two groups after 3 months of troglitazone treatment (Pro/Pro, 1.19 ± 0.17, and Ala/−, 0.93 ± 0.30 min^{-1} per μU/ml · 10^{-4}; $P = 0.46$). Furthermore, the Pro12Ala genotype did not predict change in the following values: fasting glucose (Pro/Pro, -3.9 ± 1.0, and Ala/−, -2.2 ± 1.6 mg/dl; $P = 0.35$), fasting insulin (Pro/Pro, -5.9 ± 8.5, and Ala/−, -2.5 ± 8.6 μU/ml; $P = 0.17$), acute insulin response (Pro/Pro, -60.3 ± 25.9, and Ala/−, 5.4 ± 41.2 mg/dl; $P = 0.17$), and disposition index (Pro/Pro, 297 ± 74, and Ala/−, 389 ± 125; $P = 0.53$). There were no significant changes in body weight over the 3-month period in either genotype group. Genotype did not influence changes in total cholesterol, HDL cholesterol, or triglycerides over the 3-month period (all $P \geq 0.23$).

CONCLUSIONS

We divided Hispanic women at high risk for type 2 diabetes into tertiles based on their change in insulin sensitivity during 3 months of troglitazone treatment. Women in the lowest tertile had an average response that was less than zero and, accordingly, represent women who failed to respond to the insulin-sensitizing effect of troglitazone at 400 mg/day. We previously reported that those women did not enjoy protection from type 2 diabetes during the TRIPOD study. The frequency of the common, functional Pro12Ala variant in the PPAR-γ2 gene in troglitazone nonresponders was very similar to the frequency of the variant in women who responded to the drug with modest (middle tertile) and robust (highest tertile) increases in S_i. This observation provides strong evidence that the Pro12Ala variant did not account for the prevalence of tro-glitazone nonresponders in the TRIPOD cohort. Additional analyses of continuous variables revealed a numerically smaller increase in S_i in the Ala/− subjects than in the Pro/Pro subjects, but this difference did not approach standard levels of statistical significance in this relatively small cohort. Just as important, it was very small in magnitude. The troglitazone-attendant reduction in the cumulative incidence of diabetes was similar in the two genotypes. Thus, although we cannot exclude an impact of the Pro12Ala variant on clinical or metabolic responses to tro-glitazone, our data reveal that any such effect must be very small in Hispanic women with prior gestational diabetes.

Analogous to this study, Bluher et al. (17) failed to demonstrate effects of the Ala/−/− variant on the fasting glucose and HbA$_{1c}$ responses to pioglitazone among diabetic subjects. Because an improvement in insulin action does not guarantee a clinically significant improvement in fasting glucose and HbA$_{1c}$, it is not surprising that the nonresponse rate by the definition of Bluher et al. was higher than in the present study, which defines non-response mechanistically based on insulin action. However, both Bluher et al. and we appear at odds with in vitro studies (9,10) that showed reduced binding of the Ala protein to the PPAR-responsive element of several genes and decreased transactivation in response to rosiglitazone. This in vivo/in vitro discrepancy for TZD effects is probably not due to the use of different agents in vivo and in vitro because no differences in response rates (glucose lowering) were observed among troglitazone, pioglitazone, and rosiglitazone in 127 type 2 diabetic patients who were randomly assigned to treatment with pioglitazone or rosiglitazone when troglitazone was withdrawn from clinical use (18).

What other mechanisms might account for nonresponse to troglitazone? Here we can only speculate. One possibility is that Pro12Ala is only one of a host of genetic and environmental factors influencing response. Possible additional modifiers of response to TZDs are other

genetic variants, not only in PPAR-γ2, but also in numerous associated molecules. A short list of candidates includes the retinoid X receptor-α (RXR-α), the PPAR-γ co-activator-1, lipoprotein lipase, muscle carnitine palmitoyltransferase-1, fatty acid–binding protein, and CCAAT/ enhancer-binding protein α. None of these were assessed in the present study. Variability in TZD metabolism seems less likely because estimators of systemic tro-glitazone exposure in clinical trials do not improve the prediction of response beyond that obtained based on knowledge of the administered dose (19). The clinical state of the patient, including fasting plasma glucose, HbA$_{1c}$, BMI, and fasting C-peptide, may further confound any effects of the Pro12Ala variant to an extent that statistical adjustments can only partially address (17).

Our study sample did not replicate previous reports associating the Ala/− variant with a higher insulin sensitivity, higher BMI, and protection against the development of diabetes. Although we can provide no definitive explanation for this difference, it is important to note that the TRIPOD cohort was highly unrepresentative of the population as a whole because the cohort was selected for gestational diabetes and relatively high glucose levels. Thus, effects of the Ala variant that have been observed across a range of phenotypes in the population as a whole could have been absent due to the narrow inclusion criteria.

In conclusion, we observed no evidence that isolated knowledge of a subject's Pro12Ala PPAR-γ2 genotype is useful to identify nonresponders to the insulin-sensitizing effect of troglitazone treatment in young, high-risk Hispanic women. Furthermore, while we cannot fully exclude effects of the Ala variant on insulin sensitivity or the risk of type 2 diabetes in these women, any such effects must be very small and of limited clinical significance. Thus, we conclude that isolated assessment of the Pro12Ala genotype has no apparent clinical utility in the identification of high-risk Hispanic women who will not benefit from TZD treatment.

Acknowledgments

Abbreviations

PPAR	peroxisome proliferator–activated receptor
TRIPOD	Troglitazone in Prevention of Diabetes
TZD	thiazolidinedione

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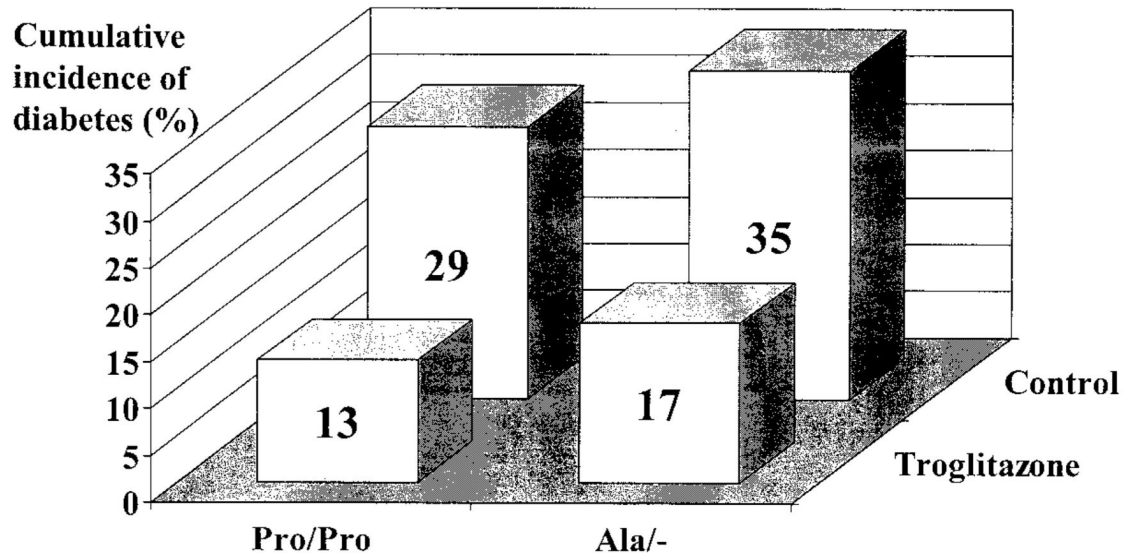
Figure 1.
Cumulative incidence of diabetes after a median follow-up of 30 months, in percentages. Data from subjects in the non-troglitazone-treated control group are also shown. The fractional cumulative incidences among those treated with troglitazone were 9/69 (Pro/Pro) and 4/24 (Ala/−). Among the control subjects, they were 12/41 (Pro/Pro) and 5/14 (Ala/−). There was no effect of genotype on the incidence of diabetes between Pro/Pro and Ala/− subjects treated with troglitazone ($P = 0.66$) or overall ($P = 0.54$).

Table 1

Mean change in S_i in response to 3 months of troglitazone treatment and corresponding genotype frequencies in women grouped into tertiles of S_i change

	Change in S_i[*]	Pro/Pro	Ala/−	Total[†]
Bottom tertile	−0.21 ± 0.57	21 (70)	9 (30)	30
Middle tertile	0.91 ± 0.26	25 (78)	7 (22)	32
Top tertile	2.58 ± 1.32	23 (74)	8 (26)	31
Total cohort	1.11 ± 1.41	69 (74)	24 (26)	93

Data are n (%) of women with each genotype or means ± SD. Observed genotype frequencies did not differ significantly from expected frequencies ($P = 0.77$ by χ^2 test).

[*] (3-month S_i) − (baseline S_i); S_i is insulin sensitivity calculated with the minimal model (in min^{-1} per μU/ml · 10^{-4});

[†] the tertiles are of unequal size because the cutoff points are based on all troglitazone-treated subjects (12), including those whose genotyping failed for technical reasons.