Current Therapeutic Research 77 (2015) 83–89

Contents lists available at ScienceDirect

Current Therapeutic Research

journal homepage: www.elsevier.com/locate/cuthre

Early Clinical Detection of Pharmacologic Response in Insulin Action in a Nondiabetic Insulin-Resistant Population

Sudha S. Shankar, MD*, R. Ravi Shankar, MD, Radha A. Railkar, PhD, Chan R. Beals, MD, PhD, Helmut O. Steinberg, MD, David E. Kelley, MD

Merck Research Laboratories, Merck & Co., Inc., Kenilworth, NJ

ARTICLE INFO

Article history:
Accepted 7 August 2015

Key words:
free fatty acids
glucose clamp
insulin resistance
insulin sensitivity
nondiabetic
pioglitazone

ABSTRACT

Background: Insulin resistance heightens the risk for type 2 diabetes mellitus and cardiovascular disease. Amelioration of insulin resistance may reduce this risk. The thiazolidinedone class of insulin sensitizers improves insulin action in individuals with insulin-resistant diabetes and nondiabetic individuals. However, there are few reports on the time of onset of such effects independent of reversal of glucotoxicity.
Objective: The goal of our study was to test whether the thiazolidinedione pioglitazone has prominent early metabolic effects that can be detected in an obese, nondiabetic, insulin-resistant population.
Methods: We conducted a randomized, double-blind, placebo-controlled, parallel-group trial in men with nondiabetic insulin resistance using a hyperinsulinemic euglycemic clamp technique (at low and high doses of insulin at 10 and 40 mU/m^2/min, respectively). The patients were given 30 mg daily oral pioglitazone or placebo for 28 days. Patients underwent a baseline clamp before initiation of treatment, and again at 14 and 28 days of treatment.
Results: Compared with placebo, under high-dose hyperinsulinemia, pioglitazone led to significant increases in glucose disposal rates (GDR) of 1.29 mg/kg/min (90% CI, 0.43–2.15; 39%; $P=0.008$) that were detectable at 2 weeks of treatment and persisted at 4 weeks of treatment. Under low-dose hyperinsulinemia, significant increases in GDR of 0.40 mg/kg/min (90% CI, 0.17–0.62; 95%; $P=0.003$) were observed at 4 weeks of treatment. These responses were accompanied by robust suppression of free fatty acids under hyperinsulinemic conditions, and by significant increases in circulating basal total adiponectin at 2 and 4 weeks of treatment.
Conclusions: Significant changes in insulin action across multiple insulin-sensitive tissues can be detected within 2 weeks of initiation of insulin-sensitizing therapy with pioglitazone in obese patients with nondiabetic insulin resistance. ClinicalTrials.gov identifier: NCT01115712.

Introduction

Insulin resistance (IR) is an integral aspect of the pathogenesis of type 2 diabetes mellitus (T2DM) and is independently associated with cardiovascular (CV) risk.[1] Amelioration of IR can be beneficial for both the prevention and treatment of T2DM.[2,3] IR can be partially normalized by exercise and weight loss, with additional contribution from pharmacologic therapy. Thiazolidinediones (TZDs) activate the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ),[4] and TZDs, besides metformin, are generally acknowledged as the only class of oral antihyperglycemic agents with therapeutically relevant effects on peripheral glucose disposal.[5] However, with the emergence of significant concerns about side effects, the use of TZDs has declined[6–8] and there is therefore an urgent need for newer insulin-sensitizing therapies.

The development of novel insulin-sensitizing therapies requires a clear understanding of the tissue site of insulin action of the novel agent, as well as the time of onset of such effects. For practical purposes, it is important to have a simple yet reliable means to perform such interrogation of insulin action early in development, and especially useful to be able to calibrate the novel treatment with the standard of care, which at the time of writing is a TZD. It is of particular interest to ascertain early responses to TZDs in individuals with nondiabetic IR because the interpretation of changes in IR would not be confounded by potential, and possibly variable, alleviation of glucotoxicity.

Various methodologies have been developed for clinical assessment of IR, including the frequently sampled intravenous glucose

* Address correspondence to: Sudha S. Shankar, MD, (Current address) Lilly Research Laboratories; Lilly Corporate Center, Drop Code 1940, Indianapolis, IN 46285.
E-mail address: sudhashankar605@gmail.com (S.S. Shankar).

http://dx.doi.org/10.1016/j.curtheres.2015.08.001

tolerance test (IVGTT)[9] and derivation of IR from an oral glucose tolerance test (OGTT) or meal tolerance test (MTT).[10] However, the gold standard methodology remains the euglycemic clamp under exogenous insulinization.[11] A number of clinical investigations have used the euglycemic clamp to examine the improvement in IR in response to TZD agents.[12–14] These studies have yielded considerable insights into the mechanisms by which PPARγ activation alters IR and glucose homeostasis, but nearly all were conducted after a treatment period of several months, when the response has presumably fully equilibrated.[3,15–17] Few studies have sought to examine leading-edge changes in IR early in the course of treatment with TZD,[18,19] and none have assessed early changes in obese human beings with nondiabetic IR in a controlled setting.

The goal of the present study was to appraise the onset of clinically relevant changes in IR in response to treatment with the TZD agent pioglitazone (PIO) using a 2-step euglycemic hyperinsulinemic clamp at 2 and 4 weeks of treatment in obese men with nondiabetic IR, with potential application of these findings as a benchmark for the evaluation of novel insulin-sensitizing therapies. Prior studies suggest that measurable changes can occur within an interval of 12 weeks[20–23]; however, none of these reported data earlier in the course of treatment. Two trials of antihyperglycemic therapy in T2DM[18,19] and a single uncontrolled trial in a small group of nonobese patients without diabetes suggested improvements as early as 3 weeks of treatment.[24] Our findings demonstrate for the first time in obese volunteers with nondiabetic IR in a randomized, double-blind, placebo-controlled setting, that improvement of IR can be clearly detected within 2 weeks of initiation of treatment with PIO. Furthermore, we demonstrate that this improvement is measurable across multiple key tissues involved in the pathogenesis of IR, including adipose, hepatic, and skeletal muscle tissues, suggesting that interorgan crosstalk likely originating from adipose tissue is already evident and is measurable systemically very early in the course of treatment.

Methods

Study Participants

All patients were overweight or obese (body mass index >28 kg/m^2 and ≤ 38 kg/m^2) men without diabetes by clinical history and fasting glucose measurement and normotensive by cuff measurements per the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI).[25] Participants also had normal cholesterol levels per the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP III) criteria,[26] and were not taking any medications routinely. All patients provided informed consent for the trial.

Study Drugs

The 30 mg PIO (Takeda Pharmaceuticals USA Inc., Deerfield, Illinois) or placebo was administered as a daily oral dose for 28 consecutive days.

Protocol

This was a randomized, double-blind, placebo-controlled study with a parallel-group design for treatment groups. The study (Protocol 170) was conducted between 2009 and 2010 at a Phase I clinical research unit (ICON Development Solutions, San Antonio, Texas) with approval from the local ethics review committee (IntegReview Ethical Review Board, San Antonio, TX). The trial was conducted in accordance with principles of Good Clinical Practice. Patients meeting study entry criteria underwent a hyperinsulinemic euglycemic clamp at baseline and were randomly allocated to 1 of 2 treatments (30 mg PIO or placebo). Patients underwent a second hyperinsulinemic euglycemic clamp after 14 days of treatment and a third hyperinsulinemic euglycemic clamp after 28 days of treatment. A total of 38 patients completed the baseline clamp procedure, and 31 and 29 patients completed the Day 14 and Day 28 clamp procedures, respectively.

The euglycemic clamp was performed in 2 steps each lasting approximately 180 minutes: a low-dose portion with insulin infusion rates of 10 mU/m^2/min followed by a high-dose portion with infusion rates of 40 mU/m^2/min. The clamp procedures were performed using the method described by DeFronzo et al[27] with target plasma glucose levels of approximately 90 mg/dL. Samples for measurement of insulin and free fatty acids (FFA) were obtained at baseline and at steady state of each step of the clamp.

The key end points of the trial were the effects of PIO compared with placebo on insulin sensitivity, measured as average changes in glucose disposal rates (GDR) from baseline at 28 days and 14 days corrected for body weight (M) at steady state of the clamp during the high- and low-dose portions. Additional end points included M/SSPG (M normalized to plasma glucose at steady state); M/I (M normalized to plasma insulin at steady state); circulating levels of fasting insulin, FFA, adiponectin, and retinol binding protein 4 (RBP4); and insulin-induced suppression of FFA. All measurements were performed using commercially available assays.

Statistical Methods

For comparison of the treatment groups with respect to the change from baseline for each end point, a constrained longitudinal data analysis method was used. This model assumes a common mean across treatment groups at baseline and a different mean for each treatment at each of the postbaseline time points. In this model, the response vector consists of baseline and the values observed at each postbaseline time point; that is, M at baseline, 14 days, and 28 days for the low- and high-dose portions of the clamp. Time is treated as a categorical variable so that no restriction is imposed on the trajectory of the means over time. The analysis model was adjusted for baseline glucose level and baseline insulin level. An unstructured covariance matrix was used to model the correlation among repeated measurements. A closed-testing procedure was employed to test the 2 primary hypotheses whereby the difference between 30 mg PIO and placebo with respect to the change in M from baseline at 28 days was tested first, and only if this hypothesis was met was the hypothesis at 14 days tested. This ensured that the overall type I error rate was controlled at the 0.05 1-sided level. One-sided confidence testing was used because the direction of the PIO effect on M is known.[28]

Further analysis was conducted to assess the reproducibility of M, insulin, and M normalized to plasma insulin at steady state (M/I) for the low- and high-dose insulin infusion clamps. Data from patients in the placebo group were used in this analysis and reproducibility was assessed over 3 time points: baseline (Day 0), Day 14, and Day 28. The concordance correlation coefficient (CCC) was computed to assess reproducibility.

Results

Overweight and obese patients with nondiabetic IR were randomly assigned in a double-blind manner to PIO and placebo (PLB) treatment groups. As shown in Table I, the 2 groups were well matched for demographic and metabolic variables, including