MODELING DISEASE PROGRESSION AND ROSIGLITAZONE INTERVENTION IN TYPE 2 DIABETIC GOTO-KAKIZAKI RATS

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Abstract

The pharmacokinetics (PK) and pharmacodynamics (PD) of rosiglitazone were studied in type 2 diabetic (T2D) Goto-Kakizaki (GK) rats which received daily doses of 0, 5 or 10 mg/kg for 23 days followed by 60-days washout. Blood glucose, plasma insulin, and hemoglobin A1c (HbA1c) were determined over time. Oral glucose tolerance tests (OGTT) were performed before and at the end of treatment, and after 20 days washout to determine insulin sensitivity and β-cell function. Rosiglitazone effectively lowered glucose by inhibiting hepatic glucose production and enhancing insulin sensitivity. The glucose-insulin inter-regulation was characterized by a feedback model: glucose and insulin have their own production \( k_{in} \) and elimination \( k_{out} \) rate constants, while glucose stimulates insulin production \( k_{inl} \) and insulin, in turn, promotes glucose utilization \( k_{outG} \). Animal handling and placebo treatment affected glucose turnover with \( k_{pl} = 0.388 \) kg/mg·day. The pharmacokinetics of rosiglitazone was fitted with a one-compartment model with first-order absorption. The effect of rosiglitazone was described as inhibition of \( k_{inG} \) with \( I_{max} = 0.296 \) and \( IC_{50} = 1.97 \) μg/ml. Rosiglitazone also stimulated glucose utilization by improving insulin sensitivity with a linear factor \( S_R = 0.0796 \) kg/mg. In GK rats, 23 days treatment increased body weight but did not cause hemodilution. Weight gain was characterized with body weight input \( k_{s}^{w} \) and output \( k_{d}^{w} \), and rosiglitazone inhibited \( k_{d}^{w} \) with an \( ID_{50} = 96.8 \) mg/kg. The mechanistic PK-PD model quantitatively described the glucose-insulin system and body weights under chronic rosiglitazone treatment in T2D rats.
Introduction

Rosiglitazone is one of the thiazolidinedione (TZD) agents which are potent agonists of the nuclear receptor Peroxisome Proliferators-Activated Receptor gamma (PPARγ) (Krentz and Bailey, 2005). The PPARγ are expressed mainly in adipose tissue, muscle, and liver. By stimulating PPARγ and subsequently modulating transcription of a series of insulin-sensitive genes, rosiglitazone improves hepatic and peripheral insulin sensitivity.

Rosiglitazone shows anti-diabetic effects in several type 2 diabetic (T2D) animals after repeated administration. In ob/ob mice, rosiglitazone treatment improved glucose tolerance and insulin sensitivity (Muurling et al., 2003). Six weeks treatment of rosiglitazone at 10 μmol/kg prevented the progression from insulin resistance to overt diabetes in Zucker diabetic fatty (ZDF) rats (Smith et al., 2000). In T2D cynomolgus monkeys, oral rosiglitazone treatment improved overall insulin regulation and significantly reduced the exogenous insulin required to maintain glycemic control (Gee et al., 2004). As an insulin sensitizer, treatment with rosiglitazone in T2D patients resulted in an improved fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c), with a concurrent reduction in insulin and C-peptide. ADOPT (A Diabetes Outcome Progression Trial) showed that rosiglitazone significantly improved insulin sensitivity over 5 years, but β-cell function was only temporarily improved during the first year of treatment (Kahn et al., 2006).

Side effects of rosiglitazone include weight gain and fluid retention (Nolan et al., 2000). Dose-related weight gain was seen with rosiglitazone alone and in combination with other hypoglycemic agents (Hollenberg, 2003). Fluid retention is typically seen as
mild hemodilution. The mechanism of weight gain is unclear, but was hypothesized to reflect increased body fat, fluid retention, and/or reduced loss of calories in the urine (Hollenberg, 2003; Semenkovich, 2005).

Goto-Kakizaki (GK) rats, a lean model of type 2 diabetes, exhibit a spontaneous polygenic disease (Goto et al., 1988). They were produced by repeated inbreeding of Wistar rats using glucose intolerance as the selection index. The diabetic state is stable after 35 generations of breeding. The GK rats show hyperglycemia, mild insulin resistance, impaired glucose-induced insulin secretion, and a decrease of β cell mass. Rosiglitazone has shown promising anti-diabetic effects in GK rats. Treatment with 14 days of 1 mg/kg rosiglitazone significantly enhanced insulin-stimulated glucose transport in adipose tissue (Kanoh et al., 2000). However, no systemic glycemic effects were observed, even when the dose was increased up to 4 mg/kg (Kanoh et al., 2001). Four weeks treatment of 20 mg/kg troglitazone or pioglitazone, the other two TZD drugs, in GK rats significantly lowered glucose and insulin concentrations (Iida et al., 2003). Thus, rosiglitazone may show glycemic benefits in GK rats with higher doses or longer treatment periods.

Mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) models can be used to quantitatively understand the relationship between drug concentrations (PK) and biological responses (Mager et al., 2003). The effect of rosiglitazone on FPG and HbA1c in T2D patients in a 26-week clinical trial was characterized by a PK/PD model, which describes the effect as stimulating glucose utilization (Benincosa and Jusko, 2003). According to simulations of this PK/PD model, rosiglitazone concentrations should remain above a threshold of twice the $SC_{50}$ for most of the dosing interval for the
maximum glucose-lowering effect. However, insulin, the important regulating hormone, was not included in this modeling effort.

Based on the anti-diabetic effects of chronic rosiglitazone treatment, we aimed to investigate the effects of repeated administration of rosiglitazone using GK rats as the animal model. The role of rosiglitazone on regulation of the glucose and insulin system and body weight was assessed using extensive experimental data and mechanistic modeling.
Methods and Materials

Animals

All studies were approved by the Institutional Animal Care and Use Committee of the University at Buffalo. Male Goto-Kakizaki rats at 9-10 weeks with weights ranging from 200-250 g were purchased from Taconic Farms (Germantown, NY). The animals had free access to food and water and were maintained on a 12/12-h light/dark cycle. All animals were acclimatized for 1 week before the initiation of the study.

Experimental

Rosiglitazone (AK Scientific Inc., Union City, CA) was dissolved immediately before oral gavage using saline for injection. Based on pre-treatment values of glucose, HbA1c, and body weight, 20 rats were divided into three groups receiving saline [placebo (P), n = 7], 5 mg/kg [low dose (L), n = 6] or 10 mg/kg [high dose (H), n = 7] of rosiglitazone solution. The treatment lasted for 23 days followed by 60 days washout. Blood (< 200 µl) was collected from the saphenous vein when glucose and insulin were required. When only glucose was monitored, a drop of blood (10 µl) was collected from the tail vein. During blood collection, the rats were under light anesthesia with 5% isofluoranes (Hospira, Inc., Lake Forest, IL). The duration of anesthesia was no longer than 5 min. All blood samples were taken between 8 to 10 am to avoid interference from circadian rhythms. The HbA1c, body weight, and hematological parameters were also monitored at various time points.

Oral glucose tolerance tests (OGTTs) were performed on Days -1 (before the start of treatment), 25 (at the end of treatment), and 42 (after 20 days of washout) to determine the insulin sensitivity and beta-cell function. At Day -1, 6 rats (randomly
chosen, \( n = 2 \) from each treatment group) received OGTTs. At Day 25 and Day 42, 3-4 rats from each group received OGTTs. In order to minimize the possible direct effects resulting from rosiglitazone and more accurately characterize the beneficial effects of the treatment in the glucose-insulin system, OGTTs were conducted on Day 25, >48 hours after the last doses of rosiglitazone. On the morning of Days -1, 25 and 42, after overnight fasting, rats received an oral dose of glucose at 2 g/kg. Glucose and insulin were measured at -5, 15 (only for glucose), 30, 45, 60 (only for glucose), 90 and 120 min. Beta-cell function \((AUC_{\text{INS/GLU}})\), insulin sensitivity indexes (Whole Body Insulin Sensitivity Index (WBISI)): \(10,000 \times \sqrt{(FG \cdot FI \times \bar{G} \cdot \bar{I})} \), Homeostasis Model Assessment Insulin Resistance (HOMA-IR): \(FG \cdot FI / 22.5 \) were calculated (Miyazaki et al., 2002), where \(AUC_{\text{INS/GLU}}\) represented the ratio of AUC of insulin profile over that of glucose profile, \(FG\) and \(FI\) represented fasting glucose and fasting insulin, and \(\bar{G}\) and \(\bar{I}\) represented average glucose and insulin levels over the duration of OGTTs.

Blood glucose was measured using a BD Logic blood glucose meter (BD Medical, Franklin Lakes, NJ) from whole blood. Plasma insulin was measured in plasma samples using a commercial rat ELISA kit (Millipore Corporation, St. Charles, MO). The assay was carried out according to manufacturer’s directions with the coefficient of variation of between assays <10%. Blood HbA1c was measured by A1cNOW InView HbA1C test meters (Metrika, Sunnyvale, CA).

A BC-2800 Veterinary Auto-hematology Analyzer (Mindray, Mahwah, NJ) was used for the hematological tests: RBC count \((10^6/\mu\text{L})\), Hb concentration \((\text{g/dL})\), hematocrit (%). Hematological parameters in blood with EDTA were analyzed within 30 min of blood collection. All procedures were based on manufacturer’s instructions.
Mechanism-Based Modeling

Figure 1 shows the PK/PD model of rosiglitazone on glucose-insulin homeostasis.

Rosiglitazone PK:

The plasma rosiglitazone concentration profiles ($C_{ROSY}$) were described as

$$C_{ROSY} = \frac{Dose \cdot F}{V \cdot (k_{el} - k_a)} \cdot (e^{-k_{at} \cdot t} - e^{-k_{el} \cdot t})$$  \hspace{1cm} (1)

where $k_a$ ( = 2.01 (L) and 8.00 (H) hr$^{-1}$ ) was the absorption rate constant, $k_{el}$ ( = 0.268 hr$^{-1}$) was the elimination rate constant, and $V$ ($V/F$=342 ml/kg) was the volume of distribution (Gao, 2011).

Glucose-Insulin Dynamics with Disease Progression:

As presented in Figure 1, the glucose ($G$) and insulin ($I$) system was characterized with two linked turnover models, which described the dynamics of these biomarkers (Lima et al., 2004; Silber et al., 2007; Jin and Jusko, 2009a; Jin and Jusko, 2009b).

$$\frac{dG}{dt} = \left( k_{inG} - k_{outG} \cdot G \cdot (1 + S_{Ins}[n] \cdot I) - k_{pl} \cdot placebo \cdot G \right) \cdot G(0) = G_0$$  \hspace{1cm} (2)

$$\frac{dI}{dt} = k_{inI} \cdot (1 + S_{G} \cdot G) - k_{out} \cdot I \cdot I(0) = I_0$$  \hspace{1cm} (3)

Glucose is constantly produced with a zero-order rate constant $k_{inG}$ and utilized with a first-order rate constant $k_{outG}$. Insulin is assumed to control glucose concentrations by stimulating its disposition with a linear efficiency constant ($S_{Ins}$). The $S_{Ins}$ represents the capability of insulin to promote glucose elimination, and is defined as insulin sensitivity. Changes of insulin sensitivity in GK rats were described by a function of $S_{Ins}$ using a series of transit compartments with an inhibition factor $k_{dis}$. Each transit compartment
was connected by $k_t$, a turnover rate constant. The equations and initial conditions
describing the first and last event compartments of $S_{\text{Ins}}$ in placebo treated animals are:

$$\frac{dS_{\text{Ins}}^P[1]}{dt} = k_i \cdot S_{\text{Ins}0} \cdot (1 - k_{\text{dix}}) - k_i \cdot S_{\text{Ins}}^P[1], \quad S_{\text{Ins}}^P[1](0) = S_{\text{Ins}0}$$

(4)

$$\frac{dS_{\text{Ins}}^P[n]}{dt} = k_i \cdot S_{\text{Ins}}^P(n-1) - k_i \cdot S_{\text{Ins}}^P[n], \quad S_{\text{Ins}}^P[2\cdot n](0) = S_{\text{Ins}0}$$

(5)

where $n$ is the number of transit compartments required to describe the changes of $S_{\text{Ins}}$ in GK rats. The $n$ was determined by trial and error and equaled 5 in the current study.

Insulin is also produced at a zero-order rate $k_{\text{inf}}$ and degraded at a first-order rate $k_{\text{out}}$. Glucose stimulates insulin production with a linear efficiency constant $S_G$, which was defined as glucose sensitivity. At time zero of the observation period, the system was assumed to be at its physiological steady-state yielding baseline equations:

$$G_0 = k_{\text{inf}} \cdot \left[ k_{\text{out}} \cdot (1 + S_{\text{Ins}0} \cdot I_0) \right]$$

(6)

$$I_0 = k_{\text{inf}} \cdot \left[ k_{\text{out}} \cdot (1 + S_G \cdot G_0) \right]$$

(7)

where initial values $G_0$ and $I_0$ were fixed as the mean glucose and insulin concentrations at time zero for each group.

The constant: $k_{\text{pl}}$ represents the placebo and/or animal handling effects observed in the control group via a hypothetical placebo compartment. The latter had a continuous input ($\text{Inp}$) of 1 unit (mg/kg) for 39 days when the intensive animal handling stopped and a first-order elimination rate constant ($k_{\text{el_p}}$):

$$\frac{d\text{Placebo}}{dt} = \text{Inp}_P(t) - k_{\text{el_p}} \cdot \text{Placebo}, \quad \text{Placebo}(0) = 0$$

with $\text{Inp}_P(t) = 1$ when $t < 39$

(8)

Effects of rosiglitazone were modeled as an inhibitory effect directly on hepatic glucose production ($k_{\text{infG}}$) via a biophase compartment ($G_{\text{eff}}$)
\[
\frac{dC_{\text{eff}}}{dt} = k_{eo} \cdot (C_{\text{ROSY}} - C_{\text{eff}}), \quad C_{\text{eff}}(0) = 0
\]  
(9)

where \(k_{eo}\) is a distribution rate constant.

Glucose in treated rats is defined as:

\[
\frac{dG}{dt} = k_{\text{in}G} \cdot (1 - \frac{I_{\text{max}} \cdot C_{\text{eff}}}{IC50 + C_{\text{eff}}}) - k_{\text{out}G} \cdot G \cdot (1 + S_{\text{Ins}}^R \cdot n) \cdot I - k_{pl} \cdot \text{placebo} \cdot G, \quad G(0) = G_0
\]

(10)

where \(I_{\text{max}}\) and \(IC50\) define the capacity and sensitivity of inhibition.

Rosiglitazone also exhibits protective effects (\(S_R\)) on insulin sensitivity:

\[
\frac{dS_{\text{Ins}}^R[1]}{dt} = k_i \cdot S_{\text{Ins}0} \cdot (1 - k_{\text{dis}}) \cdot (1 + S_R \cdot \text{Dose}) - k_i \cdot S_{\text{Ins}}^R[1], \quad S_{\text{Ins}}^R[1](0) = S_{\text{Ins}0}
\]

(11)

\[
\frac{dS_{\text{Ins}}^R[n]}{dt} = k_i \cdot S_{\text{Ins}}^R[n - 1] - k_i \cdot S_{\text{Ins}}^R[n], \quad S_{\text{Ins}}^R[2 \cdot n](0) = S_{\text{Ins}0}
\]

(12)

where \(\text{Dose} = 5\) or \(10 \text{ mg/kg}\) for time \(\leq 23\) days.

**Body Weights**

Body weight (\(WT\)) in rats under natural growth conditions can be described as:

\[
\frac{dWT}{dt} = k_w^s \cdotWT - k_d^w \cdot WT, \quad WT(0) = WT_0
\]

(13)

with \(k_w^s = k_d^w \cdot WT_{ss}\)

where \(k_w^s\) and \(k_d^w\) are the weight gain and loss rate constants, \(WT_0\) is the initial body weight, and \(WT_{ss}\) is the maximal weight that a GK rat can achieve.

Animal handling interrupts the weight gain with a hypothetical placebo dose (\(\text{Dose}_p\)) at 1 unit (mg/kg), and weight gain in placebo-treated rats is:
\[
\frac{dWT_p}{dt} = k_s^w \cdot (1 - INH_p) - k_d^w \cdot WT_p
\]

with \[INH_p = \frac{Dose_p}{ID50^w + Dose_p}\]  (14)

where \(ID50_p\) is the inhibition constant caused by animal handling.

For rosiglitazone-treated rats, body weights were affected by both animal handling and drug treatment. The possible mechanism of rosiglitazone increasing body weight by reduced loss of energy via urine (Semenkovich, 2005) was incorporated as inhibition of weight loss:

\[
\frac{dWT_r}{dt} = k_s^w \cdot (1 - INH_p) - k_d^w \cdot (1 - INH_D) \cdot WT_r
\]

with \[INH_D = \frac{Dose}{ID50^w + Dose}\]  (15)

where \(ID50^w\) is the inhibition constant of rosiglitazone for weight gain.

**Data Analysis**

Naïve-pooled data from all animals in all groups were used jointly to fit the model. Computer fittings and simulations were done using ADAPT II with the maximum likelihood method (D’Argenio and Schumitzky, 1997). The variance model was \(V_i = (\sigma_1 + \sigma_2 \cdot Y_i)^2\) where \(V_i\) is the variance of the \(i^{th}\) data point, \(\sigma_1\) and \(\sigma_2\) are the variance model parameters, and \(Y_i\) represents the \(i^{th}\) model-predicted value.

Various proposed PD models were fitted and compared. For glucose-insulin dynamics, comparison models included ones with rosiglitazone only stimulating \(k_{outG}\) or inhibiting \(k_{inG}\). The weight gain model was compared with the West model (West et al 2001) (shown in the Appendix). The final model was selected based on visual inspection of curve fittings, estimator criterion value, sum of squared residuals, Akaike...
information criterion, and confidence intervals of parameter estimations. Only final model fitting results are presented.
Results

Rosiglitazone PK

The PK of rosiglitazone in GK rats was studied previously (Gao, 2011). The simulated PK profiles under current dosing regimen using eq.1 (overlaid with previous observations) are shown in Figure 2.

Glucose-Insulin Dynamics

**Glucose.** Changes of glucose during the observation period in GK rats are shown in Figure 3. Glucose concentrations in all rats were similar at the beginning of the study (254 ± 28 mg/dL (Mean ± SD)). After initiation of the study, glucose in the control group dropped from 266 ± 24 mg/dL to 211 ± 21 mg/dL, and was maintained at this level until day 39, 16 days after the last saline dose. The treated rats also showed an initial decrease: from 260 ± 31 mg/dL to 192 ± 6 mg/dl in the 5 mg/kg group, and from 238 ± 22 mg/dL to 189 ± 17 mg/dL in the 10 mg/kg group. During the treatment period, glucose remained significantly lower in treated rats. For example, at day 14, glucose was 203 ± 23 mg/dL in the placebo group, while in the 5 mg/kg group it was 165 ± 23 mg/dL, and in the 10 mg/kg group it was 171 ± 11 mg/dL. In order to compare the overall difference, the **AUC** values during the treatment (0-23 days) were calculated. This was significantly lower in treated rats (control 4600 ± 166, 5 mg/kg 4078 ± 121, 10 mg/kg 3902 ± 89 mg·day/dL, p<0.05). The value in the 10 mg/kg group was also significantly lower than the 5 mg/kg group. Considering the possible influence of baseline differences, the average percent change from baseline in treated rats during days 14-23 was 7-10% lower than that in control rats (control: 75.1 ± 9.6%, 5 mg/kg: 64.9 ± 8.6% and 10 mg/kg: 67.9 ± 6.4%, p<0.05). Rosiglitazone dosing ceased on day
23, and after that, glucose in treated rats gradually increased to the control group level. At the end of the study, glucose in 5 mg/kg group (254 ± 75 mg/dL) was similar to that in the control group (280 ± 113 mg/dL), but glucose in the 10 mg/kg group (206 ± 14 mg/dL) was lower although not significantly (p = 0.1).

**Insulin.** Insulin concentrations in GK rats are shown in Figure 3. Insulin was not different between three groups at the beginning of the study (control: 4.26 ± 2.03, 5 mg/kg: 2.83 ± 0.54, 10 mg/kg: 3.36 ± 1.11 ng/ml). After initiation of the study, insulin in the placebo group dropped to 2.57 ± 0.67 ng/ml at day 3, and gradually increased thereafter. Insulin in the treated groups also decreased to 1.46 ± 0.40 (5 mg/kg) and 1.73 ± 0.50 (10 mg/kg) ng/ml at day 3, and remained low until the end of treatments. Insulin was lower in treated groups: at day 14, insulin was 3.24 ± 0.63 in the control group, 2.08 ± 0.40 in the 5 mg/kg group and 2.22 ± 0.56 in the 10 mg/kg group. For the overall comparison, $AUC_{0-23d}$ was significantly higher in the placebo group (P: 71.6 ± 6.7 ng·day/ml, p<0.05) than in treated groups (5 mg/kg: 46.5 ± 4.0 ng·day/ml, 10 mg/kg: 46.2 ± 6.3 ng·day/ml). Even when normalized with individual baselines, the average percent change from baseline during last 10 days in placebo group (101 ± 64%) were higher than that in treated group (5 mg/kg: 76 ± 18%, 10 mg/kg: 62 ± 13%). After the last dose, insulin in treated rats increased to the level of control rats. At the end of the study, insulin in the 5 mg/kg group (4.07 ± 2.45 ng/ml) was similar to that in the placebo group (4.41 ± 0.95 ng/ml), while insulin in the 10 mg/kg group (2.89 ± 0.83 ng/ml) was lower although not significantly (p =0.1). The decrease of Insulin after dosing was consistent with the mechanism of action of rosiglitazone as an insulin sensitizer.
OGTT. After the glucose load, similar to a literature report (Howarth et al., 2008), glucose in GK rats stayed high until 120 min, and insulin responses were shallow. The results of OGTTs are listed in Table 1. In the placebo group, fasting glucose \((FG)\) decreased after treatment, while fasting insulin \((FI)\) and AUC\(_{INS/GLU}\) did not change. The WBISI decreased over time, but not significantly. At day 25, \(FG\) was significantly higher in placebo group \((p<0.05)\), while \(FI\) and AUC\(_{INS/GLU}\) were not different. Rosiglitazone increased WBISI and decreased HOMA-IR, but not to a significant degree. On day 42, after 20 days of washout, the \(FG\) in the high dose group was significantly lower than in the low dose group \((p<0.05)\), while \(FI\) and AUC\(_{INS/GLU}\) were not different among the three groups. Control and 5 mg/kg groups had similar insulin sensitivity index values, while the 10 mg/kg group had significantly higher WBISI values \((p<0.05)\) and lower HOMA-IR values \((p<0.05)\). According to OGTT results, rosiglitazone did not show β-cell protection in GK rats and but enhanced insulin sensitivity. Furthermore, the effect on insulin sensitivity still existed 20 days after washout.

Hematology. Generally, rosiglitazone was well tolerated in GK rats. Although one common side effect is hemodilution, seen as reduced Hb and Hct, the RBC, Hb and Hct values were comparable in all groups throughout the observation period (Table 2).

HbA1c. All rats had similar HbA1c values at day 0 (Table 1). The HbA1c increased with age in control rats \((p<0.05)\), and rosiglitazone significantly decreased HbA1c. At the end of the study, the 5 mg/kg and control groups had comparable values, while the 10 mg/kg group had significantly lower values \((p<0.05)\). Consistent with the observations in our previous study (Gao et al., 2011), HbA1c in the control group increased with age, supporting the fact that the disease in GK rats was in a progressive
dynamic. In addition, since rosiglitazone did not affect Hb, the changes of HbA1c confirmed that rosiglitazone lowered the glucose in GK rats significantly.

**Modeling Analysis.** As shown in Figure 3, the present integrated PK/PD model adequately characterized glucose and insulin concentrations in GK rats over the observation period. Table 3 lists the parameter estimates. This model represented the final selection after comparing several other model versions. For example, the model with rosiglitazone only inhibiting hepatic glucose production ($k_{inG}$) failed to describe the consistently low glucose and insulin concentrations during the treatment period.

Parameters controlling glucose and insulin turnover $k_{outG}$ and $k_{outI}$ were fixed as literature values (Gao et al., 2011), which were from models with similar structures fitted to observations in GK rats. Baseline parameters $G_0$ and $I_0$ were fixed as measured basal values for each dose group. This resulted in different parameter values for the glucose ($k_{inG}$) and insulin production rate constants ($k_{ini}$), but otherwise the profiles were fitted with a universal set of parameters. The $S_{Ins0}$ was estimated as 0.995, but with a CV% 914, and therefore was fixed as 1 in the final model.

The placebo effect was handled by including a hypothetical placebo compartment with continuous infusion of a placebo dose of 1 unit (mg/kg) with a first-order elimination constant ($k_{el,p}$) similar to the rosiglitazone elimination rate constant. Rosiglitazone also contributed to the abrupt initial decrease of glucose concentration in treated rats, which was handled by direct inhibition of glucose production with drug-specific parameters $l_{max}$ and $IC_{50}$. In treated groups, plasma rosiglitazone concentrations were maintained above its $IC_{50}$ for around 8 hours after oral doses. Rosiglitazone is an insulin sensitizer, and the well-accepted mechanism of action
was reflected by modification of insulin sensitivity with a linear factor \( S_R \). The \( S_R \) was estimated as 0.0796 kg/mg, which implies that insulin sensitivity would increase 2-fold for a dose of 12.6 mg/kg.

Due to diminished effects of rosiglitazone on glucose production and insulin sensitivity and ending of intensive animal handling, glucose and insulin increased gradually after discontinuation of rosiglitazone treatment. The elevation in glucose profiles were described adequately by the model. However, the changes of insulin, especially the raise after cease of treatment at Day 23, were not well characterized. There seems to be a greater insulin increase after Day 23. We tried to include some other factors directly on insulin turn over process \( (k_{in}, \text{ and } k_{out}) \), but the model did not converge properly.

**Body Weight.** Individual body weights over time for all groups are shown in Fig. 4. The rats started at 236 ± 15 g, with no difference among the three groups. When compared with the previous study (Landersdorfer et al., 2009), the placebo rats had significantly lower \((p<0.05)\) body weights at matching ages. One possible reason could be stress from animal handling. After 3 weeks of dosing, at day 22, increases of body weight were 43.8 ± 10.8 g (Placebo), 57.9 ± 9.9 g (5 mg/kg), 61.6 ± 10.5 g (10 mg/kg), and treated groups had greater increases than the control group \((p<0.05)\). At end of the study, all rats had comparable body weights of 382 ± 21 g, as found previously.

The current model well describes the weight gain in GK rats (Figure 4). The West models could adequately fit the body weights, and the estimate for parameter \( a \) (0.38) was close to a reported value (West et al., 2001). However, CV% for estimates of \( SC_{50} \) and \( IC_{50p} \) were as high as 200%. Therefore, the current model was applied for the
characterization of body weights. The $WT_{ss}$ was fixed from previous observations (Landersdorfer et al., 2009), since the rats had comparable body weights at the end of this study.
Discussion

Rosiglitazone had been a popular insulin sensitizer before the report about cardiovascular risks (Nissen and Wolski, 2007). We investigated the effects of rosiglitazone on glucose-insulin dynamics and body weights using GK rats as the T2D model. The mechanism-based model presented here successfully characterized the glucose metabolic system in the untreated disease state and its responses to drug treatment. Rosiglitazone shows anti-diabetic effects in animals and humans. To our knowledge, this is the first study which shows that rosiglitazone reduces systemic glucose concentrations in GK rats with application of a mechanistic model to quantify this effect. In addition, the effect of rosiglitazone on body weight was also investigated. The likely mechanism of action of rosiglitazone was integrated into a model of the glucose-insulin system and a model of body weight, thereby facilitating the simultaneous analysis of drug effects.

Information about rosiglitazone in GK rats is limited. One group treated GK rats with rosiglitazone at 1 mg/kg for 14 days, and then studied the glucose uptake in isolated adipocytes (Kanoh et al., 2000). Treated rats had significantly higher glucose uptake, and the observed defect in glucose transport in GK adipocytes was no longer apparent. This report indicated that rosiglitazone exhibited a protective ability on peripheral tissue insulin sensitivity in GK rats. Later, the same group increased the dose of rosiglitazone to 4 mg/kg, but, after 14 days of treatment, plasma glucose and insulin were not affected (Kanoh et al., 2001). The author concluded that this dosing regimen was not sufficient to induce a significant glycemic effect. Two years later, another group reported that 4 weeks of 20 mg/kg troglitazone or pioglitazone significantly reduced
glucose and insulin concentrations in GK rats (Iida et al., 2003). Thus, we hypothesized that rosiglitazone would be able to decrease glucose in GK rats if we increased the dose or prolonged the treatment period. The current study demonstrated that rosiglitazone lowered glucose effectively by inhibiting hepatic glucose production and improving insulin sensitivity. Furthermore, it is of special importance that rosiglitazone showed beneficial effects even after 20 days of discontinuation of treatment.

The rats in the current study showed different glucose and insulin profiles from our previous work (Gao et al., 2011). Glucose concentrations at the beginning of the study were much lower than those in the previous study at matching ages but close to values in the literature (Adachi et al., 2003; Yasuda et al., 2003; Harris et al., 2005; Amaral et al., 2006). At Day 0, GK rats were around 9 weeks old, at which age glucose was over 300 mg/dL in the previous study. Moreover, at the end of this study (20 weeks of age), only 1 out of 7 control rats had a glucose of 507 mg/dL, while most rats had glucose over 500 mg/dL from 12 to 20 weeks of age in the previous study. In addition, the lower glucose concentrations were confirmed by HbA1c values, which were also much lower than previous observations (11.3 ± 0.87%). Furthermore, the insulin level at the beginning of this study was also half of the previous observation at the matching age. In the previous study, we observed that insulin gradually decreased and reached a much lower value at 20 weeks compared to values at 8 and 12 weeks of age. However, insulin in control rats just returned to the baseline (9 week values) in the current study. There is a possibility that we missed the insulin peak during days 39 to 84, but the exact reason for this discrepancy between the two studies is not clear.
After initiation of dosing (placebo or drug), we observed rapid declines of glucose and insulin concentrations in all rats. One possible reason could be frequent animal handling, although the rats had been acclimated for one week. However, the acute effects of animal handling are usually reported as increased glucose by inducing corticosterone (Yao et al., 2008). Nevertheless, chronic animal handling may have different patterns in influencing glucose homeostasis, and the handling effect was modeled as a direct effect on glucose homeostasis.

Because of differences observed between the current and previous study, we did not simply adapt the previous disease progression model (Gao et al., 2011). The model here describes the glucose and insulin inter-regulation with the same feedback model structure. With the additional information from the OGTTs, only changes in insulin sensitivity were included as the disease progression component.

Rosiglitazone has been modeled as stimulation of glucose utilization, with a lag time describing its delayed effect in T2D subjects (Benincosa and Jusko, 1999; Landersdorfer et al., 2009). In the current study, rosiglitazone actually showed a hypoglycemic effect as early as the first 2 to 3 days. Therefore, the lag time was not necessary, and the process of insulin sensitization was modeled as gradual improvement of insulin sensitivity.

In a disease progression model in T2D patients, pioglitazone was modeled to have disease-modifying effects on both insulin sensitivity and β-cell function (de Winter et al., 2006). However, the predicted decrease in insulin sensitivity over time was in contrast to the findings from ADOPT in recently diagnosed T2D patients, where insulin sensitivity increased under treatment with rosiglitazone (Kahn et al., 2006). In the
current study, the major mechanism of action of rosiglitazone, enhancing insulin sensitivity, with limited effects on β-cell function (Kahn et al., 2006; Deeks and Keam, 2007), were included as drug-modifying effects. As shown in Figure 5, the $S_{\text{Ins}}$ in placebo rats gradually decreased, and rosiglitazone treatment can overcome the decline of $S_{\text{Ins}}$ in a dose-dependent manner. According to model simulations, at Day 25, $S_{\text{Ins}}$ in the placebo group was 0.76 ml/ng (76% of baseline), in agreement with OGTT observations (WBISI: $1.37/1.94 = 71\%$). While WBISI increased 26% in the treated groups, the model predicted that $S_{\text{Ins}}$ was improved by 30% (5 mg/kg) and 48% (10 mg/kg). If rosiglitazone treatment lasted, $S_{\text{Ins}}$ would remain constant. After stopping dosing, $S_{\text{Ins}}$ decreased, eventually to the level of untreated rats. Model simulations demonstrated that, at Day 42, a 30% higher $S_{\text{Ins}}$ in the 10 mg/kg group than in the placebo group, which agreed well with WBISI observations (37% higher).

The model also included the effect of rosiglitazone on glucose production based on evidence from physiology and model development. It is reported that rosiglitazone reduces endogenous glucose production in type 2 diabetic patients (Miyazaki et al., 2001). In addition, troglitazone, another anti-diabetic agent in the same class, was shown to decrease glucose output rate in GK rats liver tissues (O'Rourke et al., 1997). Furthermore, the change of insulin sensitivity itself was unable to capture the glucose lowering during the early time points and the introduction of inhibition on glucose production by rosiglitazone better characterized the glucose and insulin profiles.

Rosiglitazone increased body weights in GK rats, but did not cause fluid retention. The increased body weight probably resulted from reduced energy loss via urine (Semenkovich, 2005). This effect was transient, as the body weights in all rats
were similar at the end of the study. The growth profiles of GK rats were well described by the indirect response model (Eq. 13). Landersdorfer et al. presented a mechanism-based model for body weight in GK rats: body weight was controlled by body fat, leptin concentrations and food intake (Landersdorfer et al., 2009). Admittedly, rosiglitazone was reported to increase plasma leptin in rats, and to influence body weight as well (Cai et al., 2000; Johnson et al., 2007). Since no information about these three components was collected in the current study, this model was not directly adapted.

Although our model successfully described all glucose and insulin dynamics simultaneously, it was limited by several factors. First of all, the number of transit compartments used to describe changes of insulin sensitivity was derived by the method of trial and error based on the observations in the current study, and the direct application of this number to other studies requires caution. Nevertheless, the model presented here can provide a structural model which is able to quantitatively characterize the development of insulin resistance, and the nature of transit compartment also allows flexible adjustment to the experimental observations accordingly. In addition, model complexities obliged the use of the linear stimulation coefficient $S_R$ on insulin sensitivity that could limit the predication capability. Admittedly, glucose and insulin homeostasis is controlled by multiple endogenous factors, and the effects of other hormones were not included in the model.

In conclusion, we have demonstrated that rosiglitazone effectively reduced glucose in GK rats by enhancing insulin sensitivity. The mechanistic PK/PD model adequately described the glucose and insulin changes under natural disease progression and rosiglitazone treatment. The model allows quantitation of drug effects.
on glucose-insulin homeostasis and disease progression. It could be applied to future study designs, such as assisting dose regimen selection for combination therapy in GK rats.
Authorship Contributions

Participated in research design: Gao and Jusko.

Conducted experiments: Gao.

Performed data analysis: Gao.

Wrote or contributed to the writing of the manuscript: Gao and Jusko.
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Footnotes

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The current address for Wei Gao is Clinical Pharmacology, Pfizer, Groton, CT 06340.
Legends for Figures

**Figure 1.** The PK/PD model for rosiglitazone in GK rats. Symbols are defined in the text and tables.

**Figure 2.** Time courses of rosiglitazone pharmacokinetics after 5 and 10 mg/kg doses in GK rats according to Eq. 1. Symbols in plots a) and b) represent the individual PK observations, and lines are model fittings. Lines in plot c) are simulated rosiglitazone PK profiles during the chronic dosing period.

**Figure 3.** The time course of glucose (left) and insulin (right) concentrations in the placebo (upper), 5 mg/kg (middle) and 10 mg/kg (lower) treated rats. Symbols are individual observations and lines are model fittings.

**Figure 4.** The time course of body weights for the control (left), 5 mg/kg (middle) and 10 mg/kg (right) treatment groups. Symbols are individual observations and lines are model fittings.

**Figure 5.** Simulated profiles of insulin sensitivity in GK rats for various dose regimens.
Table 1. Fasting glucose (FG), fasting insulin (FI), AUC<sub>INS/GLU</sub>, WBISI and HOMA-IR resulting from oral glucose tolerance tests in GK rats.

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of Rats</th>
<th>Group</th>
<th>FG (mg/dL)</th>
<th>FI (ng/ml)</th>
<th>AUC&lt;sub&gt;INS/GLU&lt;/sub&gt; (10&lt;sup&gt;-5&lt;/sup&gt;)</th>
<th>WBISI</th>
<th>HOMA-IR&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td></td>
<td>209 ± 12</td>
<td>1.43 ± 0.37</td>
<td>3.93 ± 0.50</td>
<td>1.94 ± 0.24</td>
<td>16.2 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Placebo</td>
<td>168 ± 18</td>
<td>1.49 ± 0.56</td>
<td>5.03 ± 1.41</td>
<td>1.37 ± 0.49</td>
<td>13.6 ± 5.7</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>Low Dose</td>
<td>146 ± 6*</td>
<td>1.17 ± 0.28</td>
<td>3.98 ± 0.74</td>
<td>1.73 ± 0.34</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>High Dose</td>
<td>142 ± 8*</td>
<td>1.33 ± 0.36</td>
<td>5.00 ± 1.87</td>
<td>1.72 ± 0.59</td>
<td>10.2 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Placebo</td>
<td>162 ± 28</td>
<td>1.46 ± 0.50</td>
<td>4.28 ± 1.10</td>
<td>1.49 ± 0.62</td>
<td>13.0 ± 5.3</td>
</tr>
<tr>
<td>42</td>
<td>4</td>
<td>Low Dose</td>
<td>177 ± 11</td>
<td>1.47 ± 0.40</td>
<td>4.85 ± 1.35</td>
<td>1.27 ± 0.46</td>
<td>14.2 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>High Dose</td>
<td>145 ± 15*</td>
<td>1.05 ± 0.32</td>
<td>3.98 ± 0.70</td>
<td>2.04 ± 0.42*</td>
<td>8.2 ± 2.7*</td>
</tr>
</tbody>
</table>

*: p<0.05 vs. Placebo

<sup>a</sup>: For HOMA-IR calculations, the glucose unit is mmol/L, and the insulin unit is μIU/ml.
Table 2. RBC, Hemoglobin (Hb), Hematocrit (Hct) and HbA1c in GK rats

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of Rats</th>
<th>Group</th>
<th>Measurement (Mean ± SD)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBC x10^6 cell</td>
<td>Hb</td>
<td>Hct</td>
<td>HbA1c</td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>Placebo</td>
<td>7.8 ± 0.4</td>
<td>15.8 ± 0.9</td>
<td>50.0 ± 2.8</td>
<td>4.94 ± 0.24</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Low Dose</td>
<td>6.6 ± 1.4</td>
<td>13.1 ± 2.9</td>
<td>42.3 ± 9.3</td>
<td>4.75 ± 0.14</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>High Dose</td>
<td>7.2 ± 0.8</td>
<td>14.4 ± 1.6</td>
<td>46.0 ± 5.3</td>
<td>4.80 ± 0.19</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>Placebo</td>
<td>8.6 ± 0.9</td>
<td>16.1 ± 1.7</td>
<td>51.1 ± 7.4</td>
<td>5.19 ± 0.14</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Low Dose</td>
<td>8.9 ± 0.2</td>
<td>16.6 ± 0.5</td>
<td>53.8 ± 1.6</td>
<td>4.95 ± 0.15 **</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>High Dose</td>
<td>8.3 ± 0.6</td>
<td>15.4 ± 1.2</td>
<td>49.8 ± 3.7</td>
<td>4.86 ± 0.27 **</td>
</tr>
<tr>
<td>42</td>
<td>7</td>
<td>Placebo</td>
<td>9.0 ± 0.7</td>
<td>16.2 ± 1.2</td>
<td>53.4 ± 3.8</td>
<td>5.41 ± 0.22</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Low Dose</td>
<td>9.0 ± 0.5</td>
<td>16.3 ± 1.2</td>
<td>53.4 ± 2.8</td>
<td>5.20 ± 0.22</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>High Dose</td>
<td>9.4 ± 0.2</td>
<td>17.0 ± 0.5</td>
<td>54.1 ± 2.2</td>
<td>5.19 ± 0.20 *</td>
</tr>
<tr>
<td>84</td>
<td>7</td>
<td>Placebo</td>
<td>9.8 ± 0.8</td>
<td>17.9 ± 0.4</td>
<td>58.1 ± 1.6</td>
<td>6.89 ± 1.61</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Low Dose</td>
<td>9.3 ± 0.8</td>
<td>16.7 ± 1.6</td>
<td>54.8 ± 4.9</td>
<td>5.86 ± 0.64</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>High Dose</td>
<td>9.8 ± 0.1</td>
<td>17.8 ± 0.2</td>
<td>57.7 ± 0.6</td>
<td>5.61 ± 0.32 *</td>
</tr>
</tbody>
</table>

** p<0.01, * p<0.05 vs. Placebo
Table 3. Pharmacodynamic parameter estimates for glucose-insulin and body weights.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Estimate (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{out}$ (day$^{-1}$)</td>
<td>Insulin output rate constant</td>
<td>408$^a$</td>
</tr>
<tr>
<td>$k_{outG}$ (day$^{-1}$)</td>
<td>Glucose output rate constant</td>
<td>66$^a$</td>
</tr>
<tr>
<td>$I_0$ (ng/ml)</td>
<td>Basal Insulin (placebo, 5 and 10 mg/kg)</td>
<td>4.02, 2.83, 3.28$^b$</td>
</tr>
<tr>
<td>$G_0$ (mg/dl)</td>
<td>Basal glucose (placebo, 5 and 10 mg/kg)</td>
<td>271, 260, 238$^b$</td>
</tr>
<tr>
<td>$S_G$ (dL/mg)</td>
<td>Glucose sensitivity</td>
<td>0.03426 (75)</td>
</tr>
<tr>
<td>$k_{dis}$</td>
<td>Disease factor on insulin sensitivity</td>
<td>0.720 (7)</td>
</tr>
<tr>
<td>$k_{el,p}$ (day$^{-1}$)</td>
<td>Placebo elimination rate constant</td>
<td>2.68 (64)</td>
</tr>
<tr>
<td>$k_{pl}$ (kg/mg·day)</td>
<td>Placebo effect constant</td>
<td>0.388 (62)</td>
</tr>
<tr>
<td>$k_{co}$ (day$^{-1}$)</td>
<td>Biophase rate constant</td>
<td>0.996 (69)</td>
</tr>
<tr>
<td>$S_R$ (kg/mg)</td>
<td>Protection factor on insulin sensitivity</td>
<td>0.0796 (20)</td>
</tr>
<tr>
<td>$IC_{50}$ (ug/mL)</td>
<td>Concentration for 50% of glucose inhibitory effect</td>
<td>1.97 (93)</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>Inhibition factor on glucose production</td>
<td>0.296 (39)</td>
</tr>
<tr>
<td>$k_{d}^w$ (1/day)</td>
<td>Body weight output rate constant</td>
<td>0.0213 (5)</td>
</tr>
<tr>
<td>$WT^s$ (g)</td>
<td>Steady-state body weight</td>
<td>398$^a$</td>
</tr>
<tr>
<td>$ID_{50p}$ (mg/kg)</td>
<td>Placebo effect constant on body weight</td>
<td>10.20 (3)</td>
</tr>
<tr>
<td>$ID_{50}$ (mg/kg)</td>
<td>Dose for 50% body weight loss inhibition</td>
<td>96.8 (16)</td>
</tr>
</tbody>
</table>

$^a$: fixed as previous observations.
$^b$: fixed as measured values.
Figure 1.
Figure 4.
Figure 5.
Appendix

West et al. (West et al., 2001) published a model for ontogenetic growth:

\[
\frac{dWT}{dt} = a \cdot WT^{-0.75} \cdot \left(1 - \left(\frac{WT}{WT_{\text{max}}}ight)^{0.25}\right)
\]

where \(WT_{\text{max}}\) is the maximal body weight. The constant \(a\) is a growth constant defined as \(B_0 \cdot m_c/E_c\), where \(B_0\) is a constant for a given taxon, \(m_c\) is the cell mass, and \(E_c\) is the metabolic energy required to create a cell. With drug treatment:

\[
\frac{dWT}{dt} = a \cdot H(t) \cdot WT^{-0.75} \cdot \left(1 - \left(\frac{WT}{WT_{\text{max}}}ight)^{0.25}\right)
\]

\[
H(t) = 1 - \frac{C_p}{IC50_p + C_p}\]

or

\[
1 + \frac{S_{\text{max}} \cdot C}{SC50 + C}
\]

where \(C_p\) is the hypothetical placebo concentration, \(ID50p\) is the inhibitory effect of animal handling, and \(S_{\text{max}}\) and \(SC50\) describe the stimulatory effects of drug on the growth factor \(a\).