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Modeling Disease Progression and Rosiglitazone Intervention in Type 2 Diabetic Goto-Kakizaki Rats

Wei Gao[1] and William J. Jusko

Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, New York

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ABSTRACT

The pharmacokinetics (PK) and pharmacodynamics (PD) of rosiglitazone were studied in type 2 diabetic (T2D) Goto-Kakizaki (GK) rats that received daily doses of 0, 5, or 10 mg/kg for 23 days followed by 60 days of washout. Blood glucose, plasma insulin, and hemoglobin A1c were determined over time. Oral glucose tolerance tests were performed before and at the end of treatment and after 20 days of 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, whereas glucose stimulates insulin production (k_{inI}) 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 PK 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 of 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 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 agents that are potent agonists of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (Krentz and Bailey, 2005). PPARγs 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 antidiabetic 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-week treatment of rosiglitazone at 10 μmol/kg prevented the progression from insulin resistance to overt diabetes in Zucker diabetic fatty 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 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

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[1]Current affiliation: Clinical Pharmacology, Pfizer, Groton, Connecticut.
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ABBREVIATIONS: PPARγ, peroxisome proliferator-activated receptor γ; GK, Goto-Kakizaki; PK, pharmacokinetics; PD, pharmacodynamics; Hb, hemoglobin; HbA1c, hemoglobin A1c; OGTT, oral glucose tolerance test; T2D, type 2 diabetic; FG, fasting glucose; FI, fasting insulin; AUC, area under the curve; WBISI, whole body insulin sensitivity index; HOMA-IR, homeostasis model assessment-insulin resistance; RBC, red blood cell; Hct, hematocrit; CV%, coefficient of variation percentage.

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 antidiabetic 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 to 4 mg/kg (Kanoh et al., 2001). Four-week treatment of 20 mg/kg troglitazone or pioglitazone, the other two thiazolidinedione 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 (PK)/pharmacodynamic (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 fasting plasma glucose 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, 1999). 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 antidiabetic effects of chronic rosiglitazone treatment, we aimed to investigate the effects of repeated administration of rosiglitazone by using GK rats as the animal model. The role of rosiglitazone on regulation of the glucose and insulin system and body weight was assessed by using extensive experimental data and mechanistic modeling.

Materials and Methods

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

Experimental Procedures. Rosiglitazone (AK Scientific Inc., Union City, CA) was dissolved immediately before oral gavage by using saline for injection. Based on pretreatment values of glucose, HbA1c, and body weight, 20 rats were divided into three groups: receiving saline (placebo; $n = 7$), 5 mg/kg rosiglitazone solution (low dose; $n = 6$), or 10 mg/kg rosiglitazone solution (high dose; $n = 7$). The treatment lasted for 23 days followed by 60 days of 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 8:00 and 10:00 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 insulin sensitivity and β-cell function. At day −1, six rats (randomly chosen, two from each treatment group) received OGTTs. At days 25 and 42, three to four rats from each group received OGTTs. 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 h after the last doses of rosiglitazone. On the mornings 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. β-Cell function ($AUC_{INS/GLU}$), insulin sensitivity indexes [whole body insulin sensitivity index (WBISI): $10{,}000/\sqrt{(FG \cdot FI)} \times (\bar{G} \cdot \bar{I})$; homeostasis model assessment insulin resistance (HOMA-IR): FG·FI/22.5] were calculated (Miyazaki et al., 2002), where $AUC_{INS/GLU}$ represents the ratio of AUC of insulin profile over that of glucose profile, FG and FI represent fasting glucose and fasting insulin, and $\bar{G}$ and $\bar{I}$ represent average glucose and insulin levels over the duration of OGTTs.

Blood glucose was measured by using a BD Logic blood glucose meter (BD Medical, Franklin Lakes, NJ) from whole blood. Plasma insulin was measured in plasma samples by using a commercial rat enzyme-linked immunosorbent assay kit (Millipore Corporation, Billerica, MA). The assay was carried out according to the manufacturer's directions with the coefficient of variation between assays <10%. Blood HbA1c was measured by using A1cNOW InView HbA1C test meters (Thermo Fisher Scientific, Waltham, MA).

A BC-2800 Veterinary Auto-hematology Analyzer (Mindray, Mahwah, NJ) was used for the hematological tests: RBC count (10^6/μl), Hb concentration (g/dl), and 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 effects on glucose-insulin homeostasis.

Rosiglitazone PK. The plasma rosiglitazone concentration profiles (C_{ROSY}) were described as

$$C_{ROSY} = \frac{\text{Dose} \cdot F}{V \cdot (k_{el} - k_a)} \cdot (e^{-ka \cdot t} - e^{-kel \cdot t}) \quad (1)$$

where k_a [= 2.01 (low) and 8.00 (high) h^{-1}] was the absorption rate constant, k_{el} (= 0.268 h^{-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 Fig. 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,b).

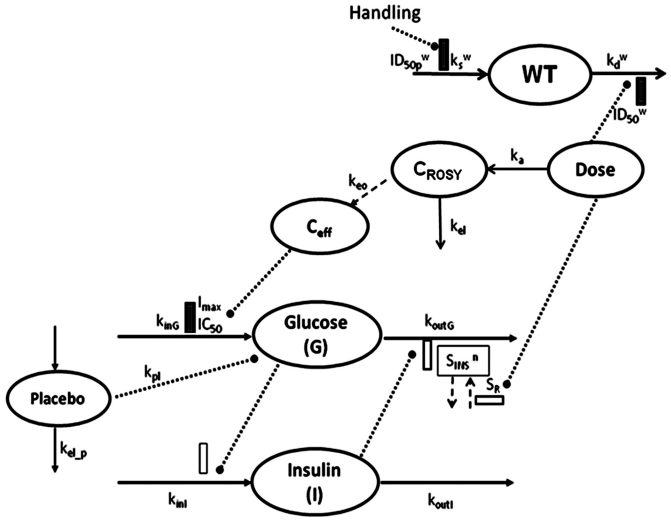

Fig. 1. The PK/PD model for rosiglitazone in GK rats. Symbols are defined in the text and tables.

$$\frac{dG}{dt} = k_{inG} - k_{outG} \cdot G \cdot (1 + S_{Ins}[n] \cdot I) - k_{pl} \cdot \text{placebo} \cdot G,$$

$$G(0) = G_0 \quad (2)$$

$$\frac{dI}{dt} = k_{inI} \cdot (1 + S_G \cdot G) - k_{outI} \cdot I, \quad I(0) = I_0 \quad (3)$$

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} by 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_{Ins} in placebo-treated animals are:

$$\frac{dS^P_{Ins}[1]}{dt} = k_t \cdot S_{Ins0} \cdot (1 - k_{dis}) - k_t \cdot S^P_{Ins}[1], \quad S^P_{Ins}[1](0) = S_{Ins0} \quad (4)$$

$$\frac{dS^P_{Ins}[n]}{dt} = k_t \cdot S^P_{Ins}[n-1] - k_t \cdot S^P_{Ins}[n], \quad S^P_{Ins}[2..n](0) = S_{Ins0} \quad (5)$$

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

Insulin is also produced at a zero-order rate k_{inI} and degraded at a first-order rate k_{outI}. Glucose stimulates insulin production with a linear efficiency constant S_G, which was defined as glucose sensitivity. At time 0 of the observation period, the system was assumed to be at its physiological steady state, yielding baseline equations:

$$G_0 = k_{inG}/[k_{outG} \cdot (1 + S_{Ins0} \cdot I_0)] \quad (6)$$

$$I_0 = k_{inI}/[k_{outI} \cdot (1 + S_G \cdot G_0)] \quad (7)$$

where initial values G_0 and I_0 were fixed as the mean glucose and insulin concentrations at time 0 for each group.

The constant k_{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 (Inp) of 1 unit (mg/kg) for 39 days when the intensive animal handling stopped and a first-order elimination rate constant (k_{el_p}):

$$\frac{d\text{Placebo}}{dt} = Inp^P(t) - k_{el\_p} \cdot \text{Placebo}, \quad \text{Placebo}(0) = 0$$

$$\text{with } Inp^P(t) = 1 \text{ when } t < 39 \text{ days} \quad (8)$$

Effects of rosiglitazone were modeled as an inhibitory effect directly on hepatic glucose production (k_{inG}) via a biophase compartment (C_{eff})

$$\frac{dC_{eff}}{dt} = k_{eo} \cdot (C_{ROSY} - C_{eff}), \quad C_{eff}(0) = 0 \quad (9)$$

where k_{eo} is a distribution rate constant.

Glucose dynamics in treated rats is:

$$\frac{dG}{dt} = k_{inG} \cdot \left(1 - \frac{I_{\max} \cdot C_{eff}}{\text{IC}^{50} + C_{eff}}\right) - k_{outG} \cdot G \cdot (1 + S^R_{Ins}[n] \cdot I) - k_{pl} \cdot \text{Placebo} \cdot G, \quad G(0) = G_0 \quad (10)$$

where $I_{\max}$ and IC_{50} define the capacity and sensitivity of inhibition.

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

$$\frac{dS^R_{Ins}[1]}{dt} = k_t \cdot S_{Ins0} \cdot (1 - k_{dis}) \cdot (1 + S_R \cdot \text{Dose}) - k_t \cdot S^R_{Ins}[1], \quad S^R_{Ins}[1](0) = S_{Ins0} \quad (11)$$

$$\frac{dS^R_{Ins}[n]}{dt} = k_t \cdot S^R_{Ins}[n-1] - k_t \cdot S^R_{Ins}[n], \quad S^R_{Ins}[2..n](0) = S_{Ins0} \quad (12)$$

where Dose = 5 or 10 mg/kg for time ≤23 days.

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

$$\frac{dWT}{dt} = k_s{}^w - k_d{}^w \cdot WT \quad WT(0) = WT_0$$

$$\text{with } k_s{}^w = k_s{}^w \cdot WT_{ss} \quad (13)$$

where k^w_s and k^w_d are the weight gain and loss rate constants, respectively, 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 (Dose_p) at 1 unit (mg/kg), and weight gain in placebo-treated rats is:

$$\frac{dWT^P}{dt} = k_s{}^w \cdot (1 - INH_p) - k_d{}^w \cdot WT^P$$

$$\text{with } INH_p = \frac{\text{Dose}_p}{\text{ID}_{50p} + \text{Dose}_p} \quad (14)$$

where ID_{50p} 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:

$$\frac{dWT^R}{dt} = k_s{}^w \cdot (1 - INH_p) - k_d{}^w \cdot (1 - INH_D) \cdot WT^R$$

$$\text{with } INH_D = \frac{\text{Dose}}{\text{ID}_{50w} + \text{Dose}} \quad (15)$$

where ID_{50w} is the inhibition constant of rosiglitazone for weight gain.

Data Analysis. Naive-pooled data from all animals in all groups were used jointly to fit the model. Computer fittings and simulations were done by 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 ith data point, σ_1 and σ_2 are the variance model parameters, and Y_i represents the ith 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 *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

regimens using eq.1 (overlaid with previous observations) are shown in Fig. 2.

Glucose-Insulin Dynamics

Glucose. Changes of glucose during the observation period in GK rats are shown in Fig. 3. Glucose concentrations in all rats were similar at the beginning of the study (254 ± 28 mg/dl; mean ± S.D.). After initiation of the study, glucose in the control group dropped from 266 ± 24 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 to 192 ± 6 mg/dl in the 5 mg/kg group and from 238 ± 22 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, whereas 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. 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 that in the 5 mg/kg group. Considering the possible influence of baseline differences, the average percentage change from baseline in treated rats during days 14 to 23 was 7 to 10% lower than that in control rats (control, 75.1 ± 9.6%; 5 mg/kg, 64.9 ± 8.6%; 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 the 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 Fig. 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 ng/ml in the control group, 2.08 ± 0.40 ng/ml in the 5 mg/kg group, and 2.22 ± 0.56 ng/ml in the 10 mg/kg group. For the overall comparison, AUC_{0-23d} was significantly higher in the placebo group (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 percentage change from baseline during the last 10 days in the placebo group (101 ± 64%) was higher than that in the 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), whereas 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.

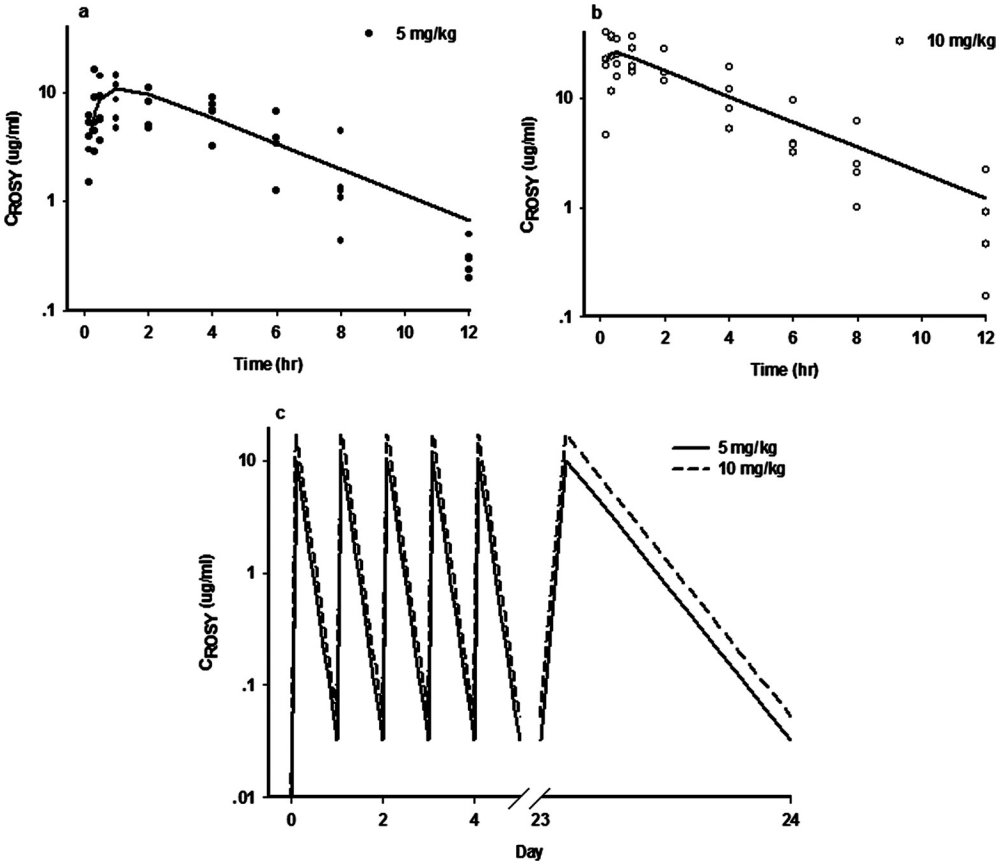

Fig. 2. Time courses of rosiglitazone pharmacokinetics after 5 (a) and 10 (b) mg/kg doses in GK rats according to eq. 1. Symbols represent the individual PK observations, and lines are model fittings. In c, lines are simulated rosiglitazone PK profiles during the chronic dosing period.

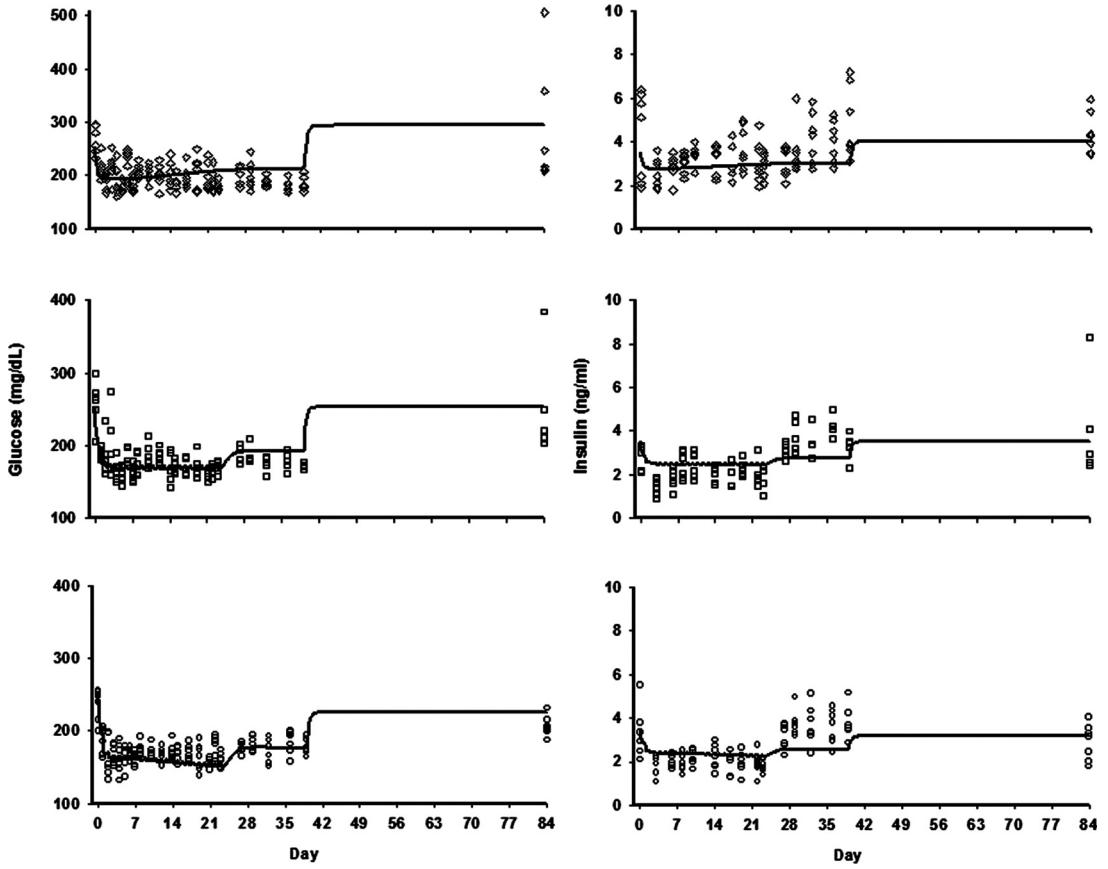

Fig. 3. The time course of glucose (left) and insulin (right) concentrations in the placebo (top), 5 mg/kg-treated (middle), and 10 mg/kg-treated (bottom) rats. Symbols are individual observations and lines are model fittings.

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, FG decreased after treatment, whereas FI and $AUC_{INS/GLU}$ did not change. The WBISI decreased over time, but not significantly. At day 25, FG was significantly higher in the placebo group ($p < 0.05$), whereas 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 ($p < 0.05$), whereas FI and $AUC_{INS/GLU}$ were not different among the three groups. Control and the 5 mg/kg groups had similar insulin sensitivity index values, whereas 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, rosgilitazone 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

TABLE 1
Parameters resulting from oral glucose tolerance tests in GK rats
Results shown are mean ± S.D.

Day	Number of Rats	Group	Measurement				
			FG	FI	$AUC_{INS/GLU}$	WBISI	HOMA-IR[a]
			mg/dl	*ng/ml*	10^{-5}		
0	6		209 ± 12	1.43 ± 0.37	3.93 ± 0.50	1.94 ± 0.24	16.2 ± 4.0
25	4	Placebo	168 ± 18	1.49 ± 0.56	5.03 ± 1.41	1.37 ± 0.49	13.6 ± 5.7
	3	Low dose	146 ± 6*	1.17 ± 0.28	3.98 ± 0.74	1.73 ± 0.34	9.8 ± 2.1
	4	High dose	142 ± 8*	1.33 ± 0.36	5.00 ± 1.87	1.72 ± 0.59	10.2 ± 2.7
42	4	Placebo	162 ± 28	1.46 ± 0.50	4.28 ± 1.10	1.49 ± 0.62	13.0 ± 5.3
	4	Low dose	177 ± 11	1.47 ± 0.40	4.85 ± 1.35	1.27 ± 0.46	14.2 ± 4.3
	4	High dose	145 ± 15*	1.05 ± 0.32	3.98 ± 0.70	2.04 ± 0.42*	8.2 ± 2.7*

[a] For HOMA-IR calculations, the glucose unit is mmol/l, and the insulin unit is μIU/ml.
* $P < 0.05$ vs. placebo.

TABLE 2
Hematology in GK rats
Results shown are mean ± S.D.

Day	Number of Rats	Group	Measurement			
			RBC	Hb	Hct	HbA1c
			$\times 10^6$ cell	g/dl	%	%
0	7	Placebo	7.8 ± 0.4	15.8 ± 0.9	50.0 ± 2.8	4.94 ± 0.24
	6	Low dose	6.6 ± 1.4	13.1 ± 2.9	42.3 ± 9.3	4.75 ± 0.14
	7	High dose	7.2 ± 0.8	14.4 ± 1.6	46.0 ± 5.3	4.80 ± 0.19
25	7	Placebo	8.6 ± 0.9	16.1 ± 1.7	51.1 ± 7.4	5.19 ± 0.14
	6	Low dose	8.9 ± 0.2	16.6 ± 0.5	53.8 ± 1.6	4.95 ± 0.15**
	7	High dose	8.3 ± 0.6	15.4 ± 1.2	49.8 ± 3.7	4.86 ± 0.27**
42	7	Placebo	9.0 ± 0.7	16.2 ± 1.2	53.4 ± 3.8	5.41 ± 0.22
	5	Low dose	9.0 ± 0.5	16.3 ± 1.2	53.4 ± 2.8	5.20 ± 0.22
	7	High dose	9.4 ± 0.2	17.0 ± 0.5	54.1 ± 2.2	5.19 ± 0.20*
84	7	Placebo	9.8 ± 0.8	17.9 ± 0.4	58.1 ± 1.6	6.89 ± 1.61
	5	Low dose	9.3 ± 0.8	16.7 ± 1.6	54.8 ± 4.9	5.86 ± 0.64
	7	High dose	9.8 ± 0.1	17.8 ± 0.2	57.7 ± 0.6	5.61 ± 0.32*

** $P < 0.01$; * $P < 0.05$ vs. placebo.

study, the 5 mg/kg and control groups had comparable values, whereas 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, because rosiglitazone did not affect Hb, the changes of HbA1c confirmed that rosiglitazone lowered the glucose in GK rats significantly.

Modeling Analysis. As shown in Fig. 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 $I_{\max}$ and IC_{50}. In treated groups, plasma rosiglitazone concentrations were maintained above its IC_{50} for approximately 8 h 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.

Because of 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 rise after cease of treatment at day 23, were not well characterized. There seems to be a greater insulin increase after day

TABLE 3
Pharmacodynamic parameter estimates for glucose-insulin and body weights

Parameter	Definition	Estimate (CV%)
k_{outI}, day^{-1}	Insulin output rate constant	408[a]
k_{outG}, day^{-1}	Glucose output rate constant	66[a]
I_0, ng/ml	Basal insulin (placebo, 5 and 10 mg/kg)	4.02, 2.83, 3.28[b]
G_0, mg/dl	Basal glucose (placebo, 5 and 10 mg/kg)	271, 260, 238[b]
S_G, dl/mg	Glucose sensitivity	0.03426 (75)
k_{dis}	Disease factor on insulin sensitivity	0.720 (7)
k_{el_p}, day^{-1}	Placebo elimination rate constant	2.68 (64)
k_{pl}, kg/mg·day	Placebo effect constant	0.388 (62)
k_{eo}, day^{-1}	Biophase rate constant	0.996 (69)
S_R, kg/mg	Protection factor on insulin sensitivity	0.0796 (20)
IC_{50}, μg/ml	Concentration for 50% of glucose inhibitory effect	1.97 (93)
$I_{\max}$	Inhibition factor on glucose production	0.296 (39)
k_d^w, day^{-1}	Body weight output rate constant	0.0213 (5)
WT_{ss}, g	Steady-state body weight	398[a]
ID_{50p}, mg/kg	Placebo effect constant on body weight	10.20 (3)
ID_{50w}, mg/kg	Dose for 50% body weight loss inhibition	96.8 (16)

[a] Fixed as previous observations.
[b] Fixed as measured values.

23. We tried to include some other factors directly on insulin turnover processes (k_{inI} and k_{outI}), 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. 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), and 61.6 ± 10.5 g (10 mg/kg), and treated groups had greater increases than the control group ($p < 0.05$). At the 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 (Fig. 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), because 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 by 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 antidiabetic effects in animals and humans. To our knowledge, this is the first study that 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 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. That 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 authors 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 approximately 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 one of seven control rats had a glucose of 507 mg/dl, whereas 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 with 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 1 week. However,

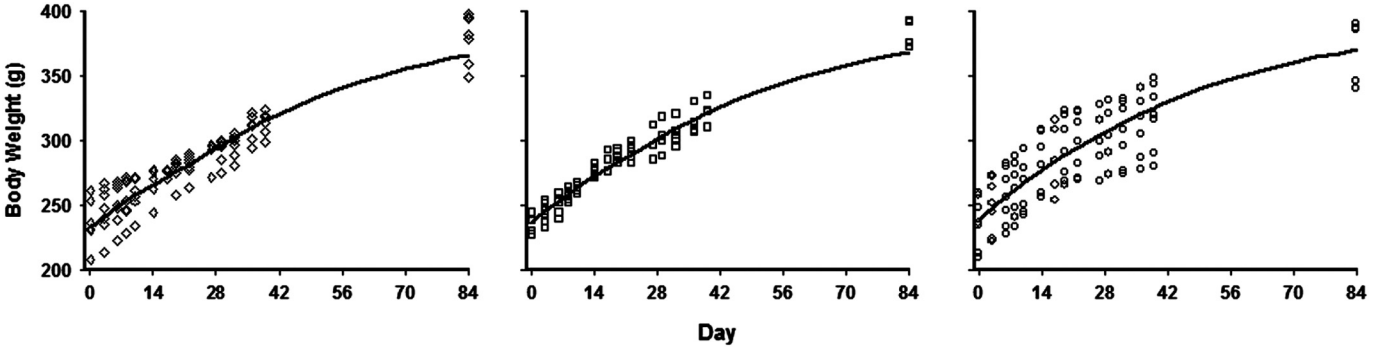

Fig. 4. The time course of body weights for the control (left), 5 mg/kg treatment (middle), and 10 mg/kg treatment (right) groups. Symbols are individual observations, and lines are model fittings.

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 (A Diabetes Outcome Progression Trial) 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 Fig. 5, the S_{Ins} in placebo rats gradually decreased, and rosiglitazone treatment can overcome the decline of S_{Ins} in a dose-dependent manner. According to model simulations, at day 25, S_{Ins} in the placebo group was 0.76 ml/ng (76% of baseline), in agreement with OGTT observations (WBISI: 1.37/1.94 = 71%). Whereas WBISI increased 26% in the treated groups, the model predicted that S_{Ins} was improved by 30% (5 mg/kg) and 48% (10 mg/kg). If rosiglitazone treatment lasted, S_{Ins} would remain constant. After stopping dosing, S_{Ins} decreased, eventually to the level of untreated rats. Model simulations demonstrated, at day 42, a 30% higher S_{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 antidiabetic 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, because 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. (2009) presented a mechanism-based model for body weight in GK rats: body weight was controlled by body fat, leptin concentrations, and food intake. Admittedly, rosiglitazone was reported to increase plasma leptin in rats and influence body weight as well (Cai et al., 2000; Johnson et al., 2007). Because 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, 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. The direct application of this number to other studies requires caution. Nevertheless, the model presented here can provide a structural model that 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

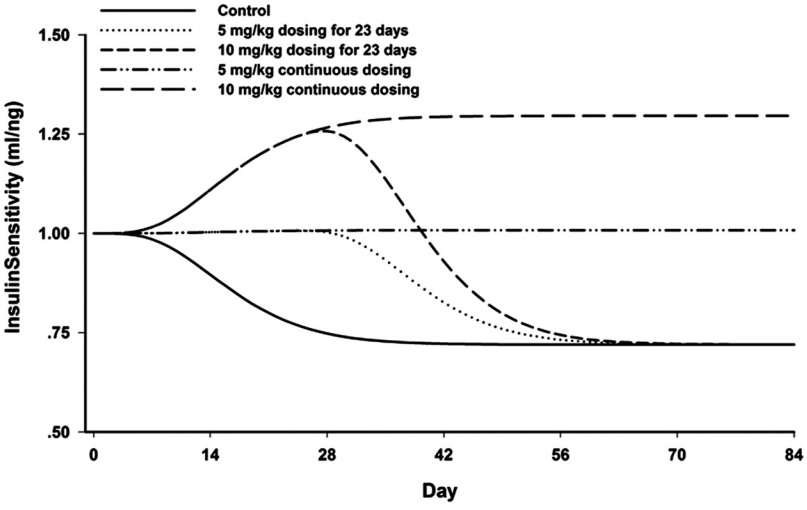

Fig. 5. Simulated profiles of insulin sensitivity in GK rats for various dosing regimens.

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.

Appendix

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

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

where $WT_{\max}$ 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:

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

$$H(t) = 1 - \frac{C_p}{IC_{50p} + C_p} \quad \text{or} \quad 1 + \frac{S_{\max} \cdot C}{SC_{50} + C}$$

where C_p is the hypothetical placebo concentration, ID_{50p} is the inhibitory effect of animal handling, and $S_{\max}$ and SC_{50} describe the stimulatory effects of drug on the growth factor a.

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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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Address correspondence to. William J. Jusko, Department of Pharmaceutical Sciences, 565 Hochstetter Hall, University at Buffalo, State University of New York, Buffalo, NY 14260. E-mail: wjjusko@buffalo.edu