Research Article

Population Pharmacodynamic Modeling of Hyperglycemic Clamp and Meal Tolerance Tests in Patients with Type 2 Diabetes Mellitus

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Abstract. In this study, glucose and insulin concentration–time profiles in subjects with type 2 diabetes mellitus (T2DM) under meal tolerance test (MTT) and hyperglycemic clamp (HGC) conditions were co-modeled simultaneously. Blood glucose and insulin concentrations were obtained from 20 subjects enrolled in a double-blind, placebo-controlled, randomized, two-way crossover study. Patients were treated with palosuran or placebo twice daily for 4 weeks and then switched to the alternative treatment after a 4-week washout period. The MTT and HGC tests were performed 1 h after drug administration on days 28 and 29 of each treatment period. Population data analysis was performed using NONMEM. The HGC model incorporates insulin-dependent glucose clearance and glucose-induced insulin secretion. This model was extended for the MTT, in which glucose absorption was described using a transit compartment with a mean transit time of 62.5 min. The incretin effect (insulin secretion triggered by oral glucose intake) was also included, but palosuran did not influence insulin secretion or sensitivity. Glucose clearance was 0.164 L/min with intersubject and interoccasion variability of 9.57% and 31.8%. Insulin-dependent glucose clearance for the HGC was about 3-fold greater than for the MTT (0.0111 vs. 0.00425 L/min/[mU/L]). The maximal incretin effect was estimated to enhance insulin secretion 2-fold. The lack of palosuran effect coupled with a population-based analysis provided quantitative insights into the variability of glucose and insulin regulation in patients with T2DM following multiple glucose tolerance tests. Application of these models may also prove useful in antihyperglycemic drug development and assessing glucose–insulin homeostasis.

KEY WORDS: hyperglycemic clamp; meal tolerance test; pharmacodynamic modeling; type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that is mainly characterized by β -cell failure and a disturbance of glucose–insulin homeostasis. Different diagnostic tests have been developed to study the regulation of glucose homeostasis and its pathological changes in diabetes. These experiments are designed to obtain an estimate of β -cell function, insulin sensitivity, and resistance. The two methods for determination of insulin secretion and sensitivity that are considered as “gold standard” are the hyperinsulinemic euglycemic glucose clamp and intravenous glucose tolerance test (IVGTT) (1).

For the euglycemic clamp, insulin is infused at a constant rate, resulting in a new steady-state insulin level that is above the fasting concentration (hyperinsulinemic). Blood glucose is frequently monitored and is “clamped”

in the normal range (euglycemic) by a glucose infusion at adjusted rates. Assuming the hyperinsulinemic state completely suppresses hepatic glucose production, the glucose infusion rate (GIR) under the steady-state clamp condition equals the glucose utilization rate (GUR). The insulin sensitivity index (S_I) can be directly determined as $GUR/(G \cdot \Delta I)$, where G is the steady-state blood glucose concentration and ΔI is the difference between fasting and steady-state plasma insulin concentration.

For IVGTT, a glucose bolus of 0.3 g/kg of body weight is administered followed by the injection of exogenous insulin (4 mU kg⁻¹ min⁻¹) or tolbutamide (100 mg) at $t=20$ min to provoke an immediate secondary peak in insulin response (2,3). Plasma glucose and insulin are frequently measured over 3 h after the glucose bolus. These data are then subjected to the minimal model analysis to generate the insulin sensitivity index (S_I) and glucose effectiveness index (S_G) (4,5). Unlike the glucose clamp method that depends on steady-state conditions, the minimal model provides estimates of insulin sensitivity, glucose effectiveness, and β -cell function using a single dynamic test. However, the minimal model oversimplifies glucose homeostasis without taking glucose–insulin mutual feedback into account. The S_I estimate may not be reliable in diabetic subjects with severe insulin resistance (6).

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The hyperglycemic glucose clamp (HGC) technique provides a highly reproducible method of assessing β -cell sensitivity to glucose following a controlled hyperglycemic stimulus (7). The increase of plasma glucose concentration to a fixed hyperglycemic plateau and maintaining it at that level are accomplished by an intravenous glucose infusion consisting of a loading dose followed by a maintenance dose adjusted by a variable GIR determined from algorithms of glucose–insulin negative feedback. As the plasma glucose concentration is held constant, the time course of the amount of glucose metabolized by the body can be quantified (1). In contrast to the IVGTT, which provokes an immediate phase of insulin secretion, the plasma insulin response to steady-state hyperglycemia is biphasic, with an early burst of insulin release followed by a phase of gradually increasing insulin concentration that lasts until the end of the study. This is particularly important because it has been suggested that loss of the initial phase of insulin secretion is the earliest detectable abnormality in T2DM (8).

The oral glucose tolerance test (OGTT) or meal tolerance test (MTT) mimics glucose and insulin dynamics under natural physiological conditions more closely than conditions of the glucose clamp and IVGTT and therefore is widely used in clinical practice for the diagnosis of T2DM. OGTT and MTT provide useful additional insight into β -cell function in that the insulin secretion stimulated by the release of incretin peptides contributes to glucose tolerance. A simple surrogate index of insulin resistance, presented as the homeostasis model assessment of insulin resistance (HOMA-IR), can be calculated as the product of the fasting glucose and fasting insulin divided by a constant (22.5) (9).

Palosuran is a nonpeptidic, orally active, potent, selective, and competitive antagonist of the human urotensin-II receptor; the concentration of which has been reported as elevated in patients with diabetes (10,11). In a rat diabetic model, palosuran showed significant beneficial effects on glycemia, serum cholesterol, triglycerides, and HbA1c (12). In addition, administration of palosuran moderately increased insulin concentrations in a significant manner (12). Based on the beneficial effect of palosuran in rat diabetic models and no remarkable safety findings in the phase I program (13), a proof-of-concept (POC) study was designed to investigate the effects of multiple-dose palosuran on insulin secretion, insulin sensitivity, and glucose levels in patients with T2DM by means of HGC and MTT techniques. The study revealed that palosuran has no effect on the study variables including first- and second-phase insulin secretion during an HGC test, insulin and blood glucose levels during a MTT, and insulin sensitivity as assessed by the HOMA-IR score (14).

The objective of this study is to apply nonlinear mixed-effects pharmacodynamic (PD) models to characterize glucose and insulin dynamic relationships under HGC and MTT conditions by taking advantage of palosuran data obtained in the POC study. The HGC and MTT models were integrated by incorporating interoccasion PD variability of the common model parameters.

METHODS

Patients and Study Design

Study participants included 16 males and 4 females with T2DM treated by diet only. The average age was 53.7 years

ranging from 40 to 65 years (SD, 7.3 years). The fasting blood glucose concentrations ranged between 110 and 180 mg/dL, and HbA1c values averaged 6.4% (range, 5.4–8.3%; SD, 0.8%). Patients were excluded if they were treated with an antidiabetic drug in the 2 months prior to screening or if they had severe concomitant diseases, more specifically diabetes complications. This study was conducted according to a protocol approved by an ethics committee for clinical research, Ethikkommission der Ärztekammer Nordrhein, Germany.

The details of the study design have been published elsewhere (14). In brief, the study was conducted as a placebo-controlled, randomized, double-blind, two-way cross-over study. The patients were treated with 125 mg palosuran or placebo twice daily for a 4-week treatment period and were switched to the alternative treatment after a 4-week washout period. Each treatment period started with the assessment of baseline and safety parameters. The MTT and HGC were performed on days 28 and 29 of treatment, respectively.

The HGC procedure was performed under fasting conditions 1 h after study drug intake on day 29 of each treatment period. Glucose was administered as an intravenous loading dose of 150 mg/kg followed by a 20% glucose solution infusion regulated by an artificial pancreas (Biostator; Life Science Instruments, Elkhart, IN) that increased the blood glucose concentration to 240 mg/dL. Blood glucose was maintained at that level for 120 min. Before starting the clamp procedure, three blood samples were obtained taken at 10-min intervals to determine the baseline glucose and insulin concentrations. The GIR and blood glucose concentrations were recorded by the Biostator on a minute-to-minute basis for the duration of the study. Serum insulin concentrations were measured every 2 min for the first 10 min and every 10 min thereafter.

The MTT was performed 1 h after study drug intake on day 28 of each treatment period. A standardized breakfast (approximately 618 kcal consisting of 65% carbohydrates, 17% proteins, and 18% lipids) was given to the patients after an overnight fasting period. Before breakfast, four blood samples were collected for the determination of baseline glucose and insulin concentrations. After breakfast, blood samples for the determination of glucose and insulin were collected at 15-min intervals over a period of 4 h.

Data Analysis

Nonlinear mixed-effects modeling was used to analyze glucose and insulin data by including established mechanisms of glucose and insulin dynamics and their feedback control. The population PD analysis was performed using first-order conditional estimation method as implemented in NONMEM (version 6; ICON Development Solutions, Ellicott City, MD). Data processing and graphical assessments were performed using S-Plus software (version 7.0; TIBCO Software Inc., Palo Alto, CA).

Model for Hyperglycemia Glucose Clamp Test

The integrated glucose–insulin model developed by Silber and colleagues (15) was used as a starting point for developing the population PD model for HGC in our study.

The model diagram of HGC is shown in Fig. 1. The glucose dynamics are described by the following differential equation:

$$\frac{dG}{dt} = GC_{ss} \cdot (CL_G + CL_{GI} \cdot IC_{ss}) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot IC_E \right) \cdot G \quad (1)$$

$$G(t=0) = GC_{ss} \cdot V_G$$

The endogenous glucose production was expressed as a function of glucose concentrations at steady-state (GC_{ss}) and the elimination rate of glucose at baseline. The elimination of glucose from the central compartment was described by the sum of two terms: the first one was insulin-independent elimination, which was proportional to glucose amount in the central compartment (G) through the rate constant calculated by insulin-independent glucose clearance (CL_G) over glucose volume of distribution (V_G), and the second one was insulin-dependent elimination, which was characterized by the product of G , the rate constant calculated by insulin-dependent glucose clearance (CL_{GI}) over V_G , and insulin concentration in an effect compartment (IC_E).

The insulin dynamics are described by the following differential equation:

$$\frac{dI}{dt} = IC_{ss} \cdot CL_I + \frac{\text{Amplitude}}{T_{dur} \cdot \sqrt{2\pi}} \cdot e^{-\frac{(t-T_{sec})^2}{2 \cdot T_{dur}^2}} + \gamma \cdot t \cdot \left(\frac{G}{V_G} - GC_{ss} \right) - \frac{CL_I}{V_I} \cdot I \quad (2)$$

$$I(t=0) = IC_{ss} \cdot V_I$$

The baseline insulin secretion was set equal to the product of the baseline insulin concentration at steady state (IC_{ss}) and insulin clearance (CL_I). The insulin secretion process includes both phases of pancreatic secretion, as some subjects showed a

distinct first- and second-phase insulin release. In the integrated glucose–insulin model proposed by Silber *et al.*, the first-phase insulin secretion was modeled as a bolus dose which was an estimated parameter. The bolus dose entered the disposition compartment through a depot compartment. This type of pulse function was not able to capture the long-lasting insulin release that probably resulted from the prolonged glucose input. We proposed a Gaussian function to account for the first phase of insulin release, where T_{sec} is the time at which maximal secretion occurs, T_{dur} is the duration of the first-phase secretion, and Amplitude is the amplitude of the first-phase insulin release (16). For the second-phase insulin secretion, the integrated glucose–insulin model used a power function to describe the regulation of glucose at non-steady state. However, this model was not able to describe the increase of second-phase insulin secretion linearly with time. As such, the rate of second-phase insulin secretion in our model was proportional to the elevation of glucose concentration at time t (G/V_G) above the threshold GC_{ss} through a constant γ . Multiplication by time, t , ensured that the rate of second-phase insulin secretion rises linearly with time. Insulin followed first-order elimination, where the rate constant is calculated as CL_I divided by insulin volume of distribution (V_I). The insulin effect compartment that regulates the insulin-dependent glucose elimination is described as:

$$\frac{dIC_E}{dt} = k_{IE} \cdot \frac{I}{V_I} - k_{IE} \cdot IC_E \quad (3)$$

$$IC_E(t=0) = IC_{ss}$$

with IC_{ss} as the insulin concentration at steady state, and k_{IE} is the rate constant of plasma insulin distributed to a “remote

Fig. 1. Hyperglycemic glucose clamp model diagram. Single compartments are shown for glucose (G) and insulin (I) along with a separate remote insulin compartment (IC_E). GP represents glucose production. CL_G and CL_{GI} are insulin-independent and insulin-dependent glucose clearance, respectively. V_G is glucose volume. IR is the first and second phase of insulin release in response to elevated glucose concentration. CL_I is insulin clearance. K_{IE} is rate constant for IC_E . The first-phase insulin release is described by a Gaussian function. The rate of second-phase insulin secretion rises linearly with time (t) and is proportional to the elevation of glucose concentration (G/V_G) above threshold GC_{ss} through a constant γ .

compartment” that has the same role as the one defined in the minimal model (4).

Model for Meal Tolerance Test

The model diagram of MTT is shown in Fig. 2. The model equations published by Jauslin *et al.* that describe an OGTT in patients with T2DM were adopted in our MTT (17). The glucose dynamics are described by differential Eq. 4. Endogenous glucose production and elimination from the glucose central compartment are described using the same functions as those defined in the HGC model (Eqs. 1 and 3).

$$\frac{dG}{dt} = \text{ABSG} + GC_{ss} \cdot (CL_G + CL_{GI} \cdot IC_{ss}) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot IC_E \right) \cdot G \quad (4)$$

$$G(t=0) = GC_{ss} \cdot V_G$$

It is a well-recognized fact that enteral glucose administration provokes greater insulin secretion than intravenous administration. This phenomenon is called the incretin effect and is primarily related to the release of glucagon-like peptide (GLP)-1. One of the important effects of GLP-1 is its inhibitory effect on gastric emptying and gastric and pancreatic exocrine secretions, which decrease glucose excursion after meals by reducing incremental glucose and insulin response (18). To account for this delayed glucose absorption, the rate of glucose absorption (ABSG) is expressed using a function that was adapted from a transit compartment absorption model (19), which was proven to be

numerically more stable and provided a physiological description of delayed absorption compared to the conventional lag-time model:

$$\text{ABSG} = \frac{k_{ca} \cdot 100,000 \cdot \text{BIO} \cdot (k_{ca} \cdot t)^n}{\sqrt{2\pi} \cdot n^{n+0.5} \cdot e^{-n}} \cdot e^{-k_{ca} \cdot t} \quad (5)$$

where k_{ca} is the transit rate constant and is parameterized in terms of the number of transit compartments (n) and the mean transit time:

$$k_{ca} = \frac{n + 1}{\text{mean transit time}} \quad (6)$$

Given the inexact glucose dose in the meal intake, a dummy dose of 100 g was introduced in the model and served as a ballpark of a standard mixed meal (20). Hence, the multiplication of the dummy dose and parameter BIO represents the amount of glucose introduced from the meal that was available in the systemic circulation.

The control mechanisms of insulin secretion from the MTT were different from the HGC. In contrast to the biphasic insulin secretion in the HGC, no first-phase insulin secretion was present in the MTT. In addition, the time course of insulin concentration mimics glucose dynamics in the MTT rather than increasing linearly with time (HGC). Therefore, the insulin dynamics are defined as the product of the baseline insulin secretion, the fraction of insulin secretion attributed to the control mechanism of plasma glucose on insulin that represents the second-phase insulin secretion on a glucose stimulus,

Fig. 2. Meal tolerance test model diagram. Single compartments are shown for glucose absorption depot (G_A), glucose central (G_C), insulin (I) along with a separate remote insulin compartment (IC_E). The absorption delay is described by transit compartment model, where k_{ca} is the transit rate constant. The incretin effect on IR provoked by glucose absorption (ABSG) was described by E_{max} model

and the fraction of insulin secretion that is driven by the incretin effect:

$$\frac{dI}{dt} = IC_{ss} \cdot CL_I \cdot \left(\frac{G}{V_G \cdot GC_{ss}} \right)^{IPRG} \cdot \left(1 + \frac{E_{max} \cdot ABSG}{ABSG + ABSG_{50}} \right) - \frac{CL_I}{V_I} \cdot I \quad (7)$$

$$I(t=0) = IC_{ss} \cdot V_I$$

A power function was used to describe the regulation of insulin secretion by the ratio of glucose concentration at time t (G/V_G) and at steady state (GC_{ss}). The IPRG is the power coefficient. The incretin effect (21) contributing to insulin secretion following the meal intake was accounted for by an E_{max} function, where E_{max} is the maximal incretin effect and $ABSG_{50}$ is the rate of glucose absorption producing 50% of the maximal incretin effect.

Palosuran Effect on Glucose and Insulin Regulation

The pharmacological properties of palosuran effect on insulin secretion and/or insulin sensitivity/resistance under HGC and MTT conditions were evaluated by Eqs. 8 and 9. The palosuran effect was parameterized as a simple dichotomous effect (presence or absence) given that only one dose level (*i.e.*, 125 mg b.i.d.) of palosuran was administered in the study and no palosuran concentrations were measured during the HGC and MTT experiments.

$$\frac{dI}{dt} = IC_{ss} \cdot CL_I + \frac{\text{Amplitude}}{T_{dur} \cdot \sqrt{2\pi}} \cdot e^{\frac{-(t-T_{sec})^2}{2 \cdot T_{dur}^2}} + \gamma \cdot t \cdot \left(\frac{G}{V_G} - GC_{ss} \right) \cdot (1 + \theta_{pal} \cdot x_i) - \frac{CL_I}{V_I} \cdot I \quad (8)$$

$$\frac{dG}{dt} = ABSG + GC_{ss} \cdot (CL_G + CL_{GI} \cdot IC_{ss}) - \left(\frac{CL_G}{V_G} + \frac{CL_{GI}}{V_G} \cdot IC_E \right) \cdot (1 + \theta_{pal} \cdot x_i) \cdot G \quad (9)$$

where θ_{pal} is the fixed effect parameter, which represents the magnitude of palosuran effect, and x_i is an indicator variable which is equal to 1 for palosuran treatment and 0 for placebo.

Random Effects Model

As the study was designed as a two-way crossover study, and the MTT and HGC were performed in the same 20 subjects, the random effects for interoccasion variability (IOV) were investigated in addition to the interindividual variability (IIV). The occasion is defined as a visit when either the HGC or MTT test was conducted. The random effects model included IIV and IOV models, which are incorporated using a log-normal random effects model specified by:

$$\theta_{ij} = \theta_{TV} \cdot \exp(\eta_i + \kappa_{ij}) \quad (10)$$

where θ_{ij} is the individual value of a PD model parameter at the j th occasion for individual i , θ_{TV} is the typical value of the

model parameter, η_i denotes the interindividual random effect accounting for the i th individual's deviation from the typical value, and κ_{ij} denotes the intraindividual random effect accounting for the i th individual deviation at the j th occasion. The η_i (κ_{ij}) terms are assumed to have a zero mean and variance ω^2 (ψ^2) (22). The IIV and IOV are reported as approximate percent coefficient of variation (%CV), calculated as:

$$\%CV(IIV) = \sqrt{\omega^2} \cdot 100\%, \quad \%CV(IOV) = \sqrt{\psi^2} \cdot 100\% \quad (11)$$

In the HGC model, IIV was estimated for CL_G , V_G , Amplitude, CL_I , V_I , and K_{IE} and was fixed to zero for T_{sec} , T_{dur} , γ , and CL_{GI_HGC} . In the MTT model, IIV was estimated for mean transit time, BIO, CL_{GI_MTT} , E_{max} , and IPRG and was fixed to zero for n and $ABSG_{50}$. IOV was included in CL_G and V_G in both models.

Residual Error Model

The residual variability was described by a log-normal residual error model:

$$\ln(Y_{ij}) = \ln(C_{ij}) + \varepsilon_{ij} \quad (12)$$

Table I. Population Parameter Estimation for Hyperglycemic Glucose Clamp Test

	Original data		Bootstrap data	
	Estimate	RSE (%)	Mean	90%CI
Structure model				
CL_G (L/min)	0.164	18.4	0.174	(0.116, 0.221)
CL_{GI_HGC} (L/min/[mU/L])	0.0111	16.6	0.0101	(0.0062, 0.0145)
V_G (L)	23.7	4.68	23.7	(22.3, 25.4)
γ (mU/[min ² mg/L])	0.000431	37.8	0.000427	(0.000202, 0.000707)
Amplitude (mU)	32.2	57.8	33.6	(10.5, 70.3)
CL_I (L/min)	1.54	44.7	1.54	(0.745, 2.78)
V_I (L) ^a	6.09	NA	NA	NA
k_{IE} (min ⁻¹)	0.00291	26.9	0.00793	(0.0014, 0.0103)
Random effects model				
IIV (%)				
CL_G	9.57	392	6.51	(0, 25.2)
V_G	15.8	37.4	15.0	(9.14, 19.4)
Amplitude	197	43.8	196	(122, 283)
CL_I	85.7	29.7	82.9	(59.1, 105)
V_I	77.6	48.5	62.8	(0, 128)
k_{IE}	90.2	51.7	82.0	(0, 132)
IOV (%)				
CL_G	31.8	45.0	29.4	(19.0, 46.2)
V_G	9.22	65.3	8.26	(1.80, 13.5)
Residual proportional error (%)				
σ_{G_HGC}	10.3	4.79	10.3	(9.54, 11.2)
σ_{I_HGC}	25.7	8.95	25.6	(22.1, 29.2)

CI confidence interval, IIV interindividual variability, IOV interoccasion variability, NA not applicable, RSE relative standard error

^a Fixed value

where Y_{ij} is the observed glucose and insulin concentration for the i th individual at time t_j , C_{ij} is the corresponding model-predicted concentration, and ε_{ij} denotes the residual random effect.

The additive error model shown as Eq. 12 was used to describe the residual error on log-transformed glucose and insulin data. Separate parameters were estimated for glucose and insulin as the residual error magnitude can be

Fig. 3. Time course of glucose and insulin concentration in representative subjects under hyperglycemic glucose clamp (HGC) condition. Subject 114 shows first-order insulin secretion. The open circle is observed value, the solid line is individual predicted concentration, and dashed line is population-predicted concentration

expected to be different between glucose and insulin. The additive error model on log-transformed data approximately corresponds to a proportional error model on non-transformed data.

Model Evaluation

The predictive performance of the population PD models was evaluated by a visual predictive check (VPC), and the reliability of

Fig. 4. Time course of glucose and insulin concentration in representative subjects under meal tolerance test (MTT) condition. The *open circle* is observed value, the *solid line* is individual predicted concentration, and *dashed line* is population-predicted concentration

population parameter estimation was assessed by a nonparametric bootstrap technique. The VPC was conducted by simulating 1,000 datasets using the population parameter estimates. The 10th, 50th, and 90th percentiles (80% VPC predictive interval) of the simulated data were constructed and superimposed on the observed data in the original analysis dataset. For the bootstrap resampling procedure, 100 bootstrap datasets were generated by being randomly sampled from the original dataset with replacement. The population PD model was fitted to the bootstrap replicates one at a time. The mean and 90% confidence interval of all the model parameters were calculated and compared with parameter values obtained from the original study.

RESULTS

The individual sets of plasma glucose and insulin concentration–time profiles under the HGC condition were modeled simultaneously using the proposed HGC model (Fig. 1). The final population parameter estimates are presented in Table I. In contrast to simply ignoring the first-phase insulin secretion, the incorporation of an empirical Gaussian function significantly stabilized the model and adequately captured the profile of first-phase insulin release. The parameters in the Gaussian function (T_{sec} and T_{dur}) were initially estimated with typical values of T_{sec} and T_{dur} of 3.27 and 0.98 min, respectively. This model achieved the global minimum but with the variance–covariance matrix aborted. Relative to the time span of the HGC test over 120 min, both the event location ($T_{\text{sec}} \sim 3.5$ min) and the width of the Gaussian first-phase secretion waveform ($T_{\text{dur}} \sim 1$ –2 min) are rather small, and, therefore, a decision was made to fix T_{sec} and T_{dur} to the literature values of 3.54 and 1.76 min,

respectively, (16) which led to the stable population PD model. The Amplitude of the Gaussian function was estimated to be 32.2 mU. The second-phase insulin release was modeled by the multiplication of γ , time, and the elevation of glucose concentration which was set to zero when the glucose concentration was equal to GC_{ss} .

The insulin volume (V_I) in the HGC model was estimated to be low (0.52 L) and was associated with large interindividual variance (7.22), which is not physiologically meaningful. Moreover, the lower estimate of V_I resulted in the biased estimation of other PD parameters. For example, the interindividual variance of Amplitude was estimated to be extremely high (26.1), and the population mean estimate of insulin clearance (CL_I) was 0.0593 L/min, which was not in agreement with the literature value of 1.22 L/min (15). Given that the physiological value of V_I has been well documented in the literature (5.11 L reported by Potocka *et al.* (23) and 9.17 L by Toffolo *et al.* (24)), the decision was made to fix V_I to 6.09 L, which had been estimated in T2DM patients using an integrated glucose–insulin model (15). The model with V_I fixed achieved successful minimization and the population PD estimates are in agreement with those reported in the literature (15).

The IIV was explored on the parameters including CL_G , V_G , Amplitude, CL_I , V_I , and k_{IE} . The introduction of IOV on CL_G and V_G led to a decrease in the objective function value by approximately 68 (−4,772 vs. −4,704 for models with and without IOV), which suggests that the inclusion of IOV on these terms resulted in a statistically significant improvement in model performance ($p < 0.001$ with 2 degrees of freedom). Representative plots of model-fitted profiles are shown in Fig. 3. In contrast to subject 104, first-phase insulin release was shown in subject 114, manifested by a rapid and

Table II. Population Parameter Estimation for Meal Tolerance Test

	Original data		Bootstrap data	
	Estimate	RSE (%)	Mean	90% CI
Structure model				
N	0.781	19.3	0.94	(0.64, 1.37)
Mean transit time (min)	62.5	10.3	66.7	(39.5, 188)
BIO	0.252	17.2	0.302	(0.18, 1.14)
$\text{CL}_{\text{GL_MTT}}$ (L/min/[mU/L])	0.00425	17.9	0.00561	(0.00267, 0.0244)
E_{max}	2.02	20.7	2.02	(1.13, 2.97)
ABSG_{50} (mg/min) ^a	14.8	NA	NA	NA
IPRG	3.06	12.5	3.17	(2.64, 4.15)
Random effects model				
IIV (%)				
Mean transit time	30.6	34.8	41.5	(18.1, 75.7)
BIO	41.5	46.1	48.9	(7.27, 78.4)
$\text{CL}_{\text{GL_MTT}}$	47.6	77.5	43.4	(0.50, 62.5)
E_{max}	56.8	50.2	70.4	(41.2, 110)
IPRG	38.9	70.2	43.1	(12.4, 64.2)
IOV (%)				
CL_G	65.7	36.6	58.8	(47.0, 72.4)
V_G	19.5	36.0	21.7	(15.5, 27.9)
Residual proportional error (%)				
$\sigma_{\text{G_MTT}}$	7.02	6.77	6.82	(6.13, 7.50)
$\sigma_{\text{L_MTT}}$	30.2	6.82	29.6	(27.1, 32.2)

CI confidence interval, IIV interindividual variability, IOV interoccasion variability, NA not applicable, RSE relative standard error

^a Fixed value

significant increase in insulin concentration upon the initiation of the HGC procedure. The effect of palosuran on second-phase insulin secretion was assessed. The typical value of the effect magnitude was estimated to be -0.122 with its

95% confidence interval including zero, suggesting palosuran did not enhance glucose-regulated insulin secretion.

The MTT model (Fig. 2) was fit to the glucose and insulin data under the MTT condition. The model-fitted

Fig. 5. Visual predictive check of hyperglycemic clamp model. One thousand datasets were simulated from the original dataset and the model parameter estimates. The lines represent the 10th, 50th, and 90th percentiles

profiles in the representative individuals are shown in Fig. 4. The population parameters including CL_G , V_G , CL_I , V_I , and k_{IE} were fixed to those estimated from the HGC model with the assumption that these disposition parameters should not

markedly differ between HGC and MTT after the incretin effect had been taken into account. Palosuran was estimated to decrease glucose elimination by 7.44% which was not considered to be clinically relevant. In light that palosuran did

Fig. 6. Visual predictive check of the meal tolerance test model. One thousand datasets were simulated from the original dataset and the model parameter estimates. The lines represent the 10th, 50th, and 90th percentiles

not influence insulin secretion and insulin sensitivity in a clinically meaningful manner, the HGC and MTT models were fit to the entire data under both conditions with the treatment effect of palosuran set to zero. The population parameters relevant to the MTT were re-estimated and listed in Table II. The IOV of CL_G and V_G now reflects intraindividual random variability between four occasions.

The glucose absorption delay was described by a chain of transit compartments through which the glucose intake entered the central compartment (19). The mean transit time, which represents the average time for an orally administered glucose molecule to be absorbed in the central compartment, was 62.5 min. The incretin effect, which is the release of the incretin peptide hormones provoked by meal digestion, was modeled by an $E_{\max}$ model, where $E_{\max}$ was estimated to be 2.02. The glucose absorption rate producing 50% of maximum incretin effect ($ABSG_{50}$) cannot be identified by the model, which may be explained by the correlation between $E_{\max}$ and $ABSG_{50}$. Given that the scale of insulin concentration was comparable to the one presented in the literature, $ABSG_{50}$ was fixed to the literature value (14.8 mg/min) (17). A sensitivity analysis was further conducted to assess the impact of $ABSG_{50}$. Fixing $ABSG_{50}$ to 0.148 mg/min (100-fold lower than the literature value) did not significantly change the fixed and random effects parameter estimates (bias within 25%) except for n and mean transit time (1.42 and 44.2 min vs. 0.781 and 62.5 min).

During the MTT model development, a glucose effect compartment was introduced to account for the delayed glucose stimulation effect on insulin release. Although the model attained good parameter estimation and described the data well, the rate constant associated with glucose effect compartment (k_{GE}) was estimated to be high (1.56 min^{-1}), suggesting that glucose concentrations in the central and effect compartment achieved rapid equilibrium. The VPC plot for the HGC and MTT models shows that model predictions are largely in agreement with the observed data (Figs. 5 and 6).

DISCUSSION

The present study extends the published modeling framework for glucose and insulin (15,17), and the lack of therapeutic effects of palosuran on insulin secretion and insulin sensitivity/resistance essentially provided an assessment of IOV under multiple glucose tolerance test conditions. The possible antidiabetic mechanisms of palosuran included enhancing insulin secretion and/or insulin sensitivity. These drug actions were originally incorporated into the integrated glucose–insulin models, thereby facilitating the simultaneous analysis of glucose and insulin responses to perturbations (hyperglycemia and meal intake) and drug effects. Given the absence of drug concentrations measured in the study, the drug effect was estimated in the model as a dichotomous factor.

In general, glucose and insulin exerted a coordinated effect on hepatic glucose production (25). However, differentiation of the insulin effect from the glucose effect was often difficult as glucose and insulin concentrations were correlated, reflected by increasing insulin concentrations in response to glucose provocation. Silber and colleagues estimated the glucose effect on glucose production and found that further incorporating an insulin effect did not improve the goodness-of-fit (15). Moreover, the feedback control on glucose production was estimated to be close to zero in patients with T2DM (15). As such, constant glucose production was assumed in our model. The other modeling aspect that differed from the published models was the functions used to describe insulin secretion. The first-phase insulin secretion under the HGC condition was captured by an empirical Gaussian function in our model, whereas it was either estimated as a bolus dose for healthy subjects or assumed to be absent in patients with T2DM in the published models (15,17). The second-phase insulin secretion was generally considered to be stimulated by the elevation of glucose concentration above its steady-state level (26). Thus, the

Fig. 7. Comparison of total glucose clearance (median) between placebo and palosuran treatment during hyperglycemic glucose clamp (HGC) test and meal tolerance test (MTT). Total glucose clearance is calculated as the sum of insulin-independent glucose clearance (CL_G) and multiplication of insulin-dependent glucose clearance (CL_{GI}) and insulin concentration in the effect compartment (IC_E)

difference between glucose concentration at time t (G/V_G) and glucose at steady state (GC_{ss}) was used as a driving force in our HGC model, whereas a power function with exponent of G/V_G over GC_{ss} was used in the published models (15,17).

The population PD parameter estimates controlling glucose and insulin dynamics in our model are in accordance with those literature values despite some discrepancies in the functions that were used to describe the control mechanisms of glucose and insulin. For example, the insulin-independent glucose clearance (CL_G), insulin-dependent glucose clearance under the HGC condition (CL_{GL_HGC}), insulin-dependent glucose clearance under the MTT condition (CL_{GL_MTT}), and insulin clearance (CL_I) were estimated to be 0.164 L/min, 0.0111 L/min/[mU/L], 0.00425 L/min/[mU/L], and 1.54 L/min, respectively, which were in proximity to published values (0.0894 L/min, 0.00829 L/min/[mU/L], 0.0059 L/min/[mU/L], and 1.22 L/min) (15,17). Furthermore, the maximal incretin effect, which was attributed to incretin hormones (*e.g.*, GLP-1) secreted in response to nutrient intake in the gut, was 2.02 in our model *vs.* 1.47 published previously (17).

A POC study was conducted to investigate the clinical effects of palosuran on insulin secretion and sensitivity on the basis of results from animal studies showing that palosuran improved pancreatic function and reduced HbA1c levels (12,14). Patients were treated with palosuran 125 mg or placebo twice daily for a 4-week treatment period and, after a 4-week washout period, switched to the alternative regimen. In a study with T2DM nephropathy patients, oral doses of palosuran 125 mg b.i.d. significantly reduced the 24-h urinary albumin excretion rate (27). Furthermore, Clozel *et al.* showed a significant decrease in urotensin-II-induced contraction at 1 μ M palosuran in a rat assay that assessed the functional selectivity of palosuran for the urotensin-II receptor (UT receptor) (10). Taking into account the 10-fold difference in inhibitory potency on the human compared with the rat UT receptor, this would translate into a concentration of approximately 50 ng/mL, which was well covered by the concentrations found in patients in this study. Therefore, a dose of 125 mg b.i.d. was chosen which was considered sufficient to investigate the objectives of this study.

The change in the second-phase insulin response under the HGC condition was chosen as the primary efficacy endpoint in this study, which was calculated as the difference from baseline in the incremental insulin response during the last hour of the HGC procedure. The mean second-phase insulin response was 37.5 and 39.3 μ U mL⁻¹ for palosuran and placebo, respectively, and the difference between the treatments was -1.8 with 95% confidence interval including zero. The insulin sensitivity was also investigated as assessed by HOMA-IR score during 28 days of treatment. Palosuran showed no effect on this secondary efficacy parameter. The lack of palosuran effect on insulin and glucose regulation in a diet-treated diabetic patient population is in contrast with the beneficial effects of palosuran observed in the rat diabetic model. The underlying reasons for the discrepancy between animal and human observations are unknown. One possible explanation is that urotensin-II and its receptor have different functions in humans, which may warrant more research to understand its precise function.

The lack of clinical beneficial effect of palosuran on improving insulin secretion and insulin sensitivity was further supported by the integrated glucose-insulin models, in which the palosuran treatment effect was incorporated as shown in Eqs. 8 and 9, and the effect magnitude was quantified to be not clinically meaningful. Furthermore, the model-predicted total glucose clearance was calculated as the sum of the insulin-independent glucose clearance (CL_G) and multiplication of insulin-dependent glucose clearance (CL_{GI}) and insulin concentration in the effect compartment (IC_E). The median value of total glucose clearance *vs.* time was plotted for each treatment and each test (Fig. 7). No evidence has shown that palosuran enhanced glucose clearance under the HGC and MTT conditions.

CONCLUSIONS

Population PD modeling was applied to evaluate regulation and variability of glucose and insulin in T2DM following HGC and MTT procedures in a quantitative manner. Modeling results suggest that palosuran was not superior to placebo in enhancing the insulin secretion and insulin sensitivity/resistance. Interestingly, the IOV in glucose utilization appears greater than IIV, and the models and parameter values may prove useful in the design and analysis of studies conducted to assess the effect of antidiabetic compounds on glucose-insulin feedback.

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Conflict of Interest Drs. Dingemanse and Sidharta are employed by Actelion Pharmaceuticals Ltd. Drs. Hong and Mager report no conflict of interest.

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