Research Article

A Semi-mechanistic Model for the Effects of a Novel Glucagon Receptor Antagonist on Glucagon and the Interaction Between Glucose, Glucagon, and Insulin Applied to Adaptive Phase II Design

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Abstract. A potent novel compound (MK-3577) was developed for the treatment of type 2 diabetes mellitus (T2DM) through blocking the glucagon receptor. A semi-mechanistic model was developed to describe the drug effect on glucagon and the interaction between glucagon, insulin, and glucose in healthy subjects ($N=36$) during a glucagon challenge study in which glucagon, octreotide (Sandostatin), and basal insulin were infused for 2 h starting from 3, 12, or 24 h postdose of a single 0–900 mg MK-3577 administration. The drug effect was modeled by using an inhibitory $E_{\max}$ model ($I_{\max}=0.96$ and $IC_{50}=13.9$ nM) to reduce the ability of glucagon to increase the glucose production rate (GPROD). In addition, an $E_{\max}$ model ($E_{\max}=0.79$ and $EC_{50}=575$ nM) to increase glucagon secretion by the drug was used to account for the increased glucagon concentrations prechallenge (*via* compensatory feedback). The model adequately captured the observed profiles of glucagon, glucose, and insulin pre- and postchallenge. The model was then adapted for the T2DM patient population. A linear model to correlate fasting plasma glucose (FPG) to weighted mean glucose (WMG) was developed and provided robust predictions to assist with the dose adjustment for the interim analysis of a phase IIa study.

KEY WORDS: glucagon challenge; glucagon receptor antagonist; glucose and insulin; modeling and simulation; type 2 diabetes.

INTRODUCTION

The regulation of glucose homeostasis is multifaceted (1–3). In healthy subjects, elevated plasma glucose concentrations result in an increase of glucose in the pancreatic islets,

triggering insulin secretion from the β cells and decreasing the glucagon secretion from the α cells in the vicinity. Consequently, glucagon released from the pancreatic islets into the portal vein decreases and reduced liver glucagon concentration decreases liver glucose production. In addition, insulin stimulates glucose utilization in insulin-sensitive tissues such as fat and muscle. Glucose uptake by the brain is highly preserved as an essential functionality and is insulin-independent.

Type 2 diabetes mellitus (T2DM) is a world health concern (4,5). The American Diabetes Association currently recommends a treatment target for A1C of <7.0%. However, many patients with T2DM are unable to achieve these targets with contemporary therapies. The significance of the role of excessive glucagon action in the pathophysiology of hyperglycemia in T2DM has become increasingly apparent (6,7). Two approaches to reducing the effect of glucagon in diabetes have been explored in man—therapies that reduce glucagon secretion and therapies that block glucagon effects at the receptor level. Somatostatin, which inhibits secretion of both insulin and glucagon, has been demonstrated to be effective in insulin-deficient type 1 diabetic patients, supporting the concept that glucagon action is a relevant target for antihyperglycemic therapy (8).

There are several mathematical models developed to describe the regulation loop for glucose homeostasis (9–13). These models focus on the interaction between glucose and

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insulin, but have left out the glucagon effect, presumably because glucagon levels were not regularly measured in these studies. Another model describing the feedback relationship between glucose and glucagon in the presence of a monoclonal antibody antagonist of glucagon receptor in mice was developed, but insulin was not collected and incorporated in the model due to the limitation of maximum volume of blood collection (14). Recently, an integrated glucose-insulin-glucagon model was published for an oral glucokinase activator (15). There are also physiologically based (system biology) models of the glucose-insulin-glucagon regulatory system that utilize anatomical organ and tissue compartments, blood flow, exchange between compartments, metabolic processes, etc., which require good knowledge of different pathways and system parameters to provide robust prediction (16,17).

MK-3577, a potent novel glucagon receptor antagonist blocking the glucagon effect, was developed for the treatment of T2DM as an oral compound. It inhibits glucagon-mediated cyclic adenosine monophosphate (cAMP) increases in Chinese hamster ovary (CHO) cells expressing the human glucagon receptor with a half-maximal inhibitory concentration value (IC_{50}) of 8.3 nM. One important aspect of proof of pharmacology for this compound was to evaluate whether sustained 24 h or transient blockade of the glucagon effect would be necessary to achieve clinically important glucose-lowering efficacy.

The objectives of this work were (1) to develop a semi-mechanistic model to describe the drug effect on glucagon and the interaction between glucagon, insulin, and glucose in a first-in-man (FIM) study in healthy subjects where glucagon challenge was conducted and (2) to adapt the model for the T2DM patient population and utilize clinical trial simulation (CTS) to aid dose adjustment for the interim analysis of a phase IIa study in T2DM patients.

MATERIAL AND METHODS

Subjects and Study Design

For both the FIM and phase IIa studies, the Institutional Review Board/Ethical Review Committee at each site approved the protocol and all subjects gave written informed consent prior to study entry. The studies were conducted in accordance with the Declaration of Helsinki and ICH Guideline for Good Clinical Practice. The baseline characteristics of the subjects who were enrolled in these two studies are shown in Table I.

FIM Study (Glucagon Challenge Study) in Healthy Subjects

This was a randomized, double-blind, placebo-controlled, crossover study with a balanced incomplete block design (Table II). A total of 36 healthy male subjects were randomized to receive various single dose treatments of placebo and MK-3577 ranging from 1 to 900 mg either in the morning or in the evening. At 3, 12, or 24 h postdose, a 2-h infusion of glucagon (3 ng/kg/min), octreotide (Sandostatin, 30 ng/kg/min), and insulin (0.1 mIU/kg/min) was carried out. Sandostatin is a somatostatin analog which blocks the normal endogenous insulin and glucagon secretions. A basal insulin infusion was given to reduce the degree of excessive glycemia.

There was at least a 5- to 7-day minimum washout interval between the administrations of study drug in each period.

Blood samples were obtained predose and up to 14 h postdose in parts II and III and up to 32 h postdose in part IV to determine MK-3577 plasma concentrations. Glucose, glucagon, and insulin concentrations were also measured.

Phase IIa Study in T2DM Patients

Approximately 276 T2DM patients were to be randomized in this multicenter, double-blind, placebo- and active-controlled, four-period/five-treatment incomplete cross-over study. Treatments consisted of MK-3577 10 mg QD in the AM, MK-3577 6 mg QD in the PM, MK-3577 25 mg BID, metformin 1,000 mg BID (active control), or placebo.

A prespecified interim analysis was performed after the first 118 patients completed the first two periods of the study to assess the glucose-lowering efficacy and safety profile of MK-3577, and guide potential dose adaptation.

Prior to the interim analysis, modeling and simulation were conducted, the results of which were used to trigger drug material production if the selected doses for the ongoing phase IIa study were deemed too low. Making early and accurate decisions about drug material production would save time and reduce cost.

Analytical Methods

A central lab (PPD) was used to analyze glucose, glucagon, and insulin samples. MK-3577 plasma samples were analyzed by Merck Bioanalysis group. The linear calibration range for MK-3577 assay was 5–2,000 ng/mL, with 5 ng/mL defined as the lowest limit of quantitation. The overall interday assay precision and mean inaccuracy was $\leq 3.34\%$ and $\leq 1.70\%$, respectively.

Modeling Method for MK-3577 Population Pharmacokinetics

A one-compartment model with first-order absorption was used to describe MK-3577 concentration profiles. The relative bioavailability of MK-3577 for pm dosing was estimated compared to am dosing. The elimination of MK-3577 was found to follow a circadian rhythm. A cosine function was used to describe this circadian rhythm (Eq. 1).

$$k_d = k_{\min} + (k_{\max} - k_{\min}) \times \left(\frac{\cos\left((T + \text{NDI} \times 12 - T_{\text{KM}}) \times 3.14 \times 2 / 24\right)}{2} + 0.5 \right) \quad (1)$$

where k_d is the elimination rate constant from the central compartment, $k_{\min}$ and $k_{\max}$ are the minimal and maximal elimination rate constants, respectively, T is the time after MK-3577 dose which is set as time 0, NDI is a night dose indicator, and T_{KM} is the time after the am dose of the maximal elimination rate constant. NDI=0 if the dose is given in am, and NDI=1 if the dose is given in pm.

Table I. Baseline Characteristics for the FIM and Phase IIa Studies

Characteristic	FIM (<i>N</i> =36)	Phase IIa (<i>N</i> =118 ^a)
Age, years	35±7.1	54±10
Gender, males, <i>n</i> (%)	36 (100)	63 (53)
Body mass index, kg/m ²	24.7±2.5	29.4±5.6
Duration of T2DM, years ^b	0	3
A1c, %	–	7.6±0.8
FPG, mg/dL	93±8.3 ^c	152±35
2-h PMG, mg/dL	–	223±63

Data are mean±SD, number of subjects (proportion), or otherwise noted

A1c and 2-h PMG baseline values were not collected in the FIM study because these were healthy subjects

A1c hemoglobin A1c, FPG fasting plasma glucose, PMG postmeal glucose

^a For a prespecified interim analysis

^b Median

^c *N*=24 because FPG baseline was not collected for the 12 subjects in part 3. Mean±standard deviation (SD) FPG at 6 am (which was 10 h post MK-3577 dose and 2 h prior to the glucagon challenge) in these 12 subjects were 83±6.9 mg/dL

Modeling Method for Healthy Subjects with Glucagon Challenge

A published model for intravenous glucose tolerance test by Silber *et al.* (9) was adopted with modifications. Figure 1a shows the model structure for MK-3577's effect on glucagon, and the interaction between glucagon, glucose, and insulin in healthy subjects treated with glucagon challenge. A few modifications from Silber's model were made to better describe the glucagon challenge data.

Firstly, glucagon was explicitly included in the current model, rather than implicitly embedded in the glucose self-inhibitory effect on its own production rate (GPROD) in Silber's model. This was necessary for the updated model because the drug effect was on the glucagon receptors. Intense sampling of glucagon enabled a quantitative estimation of glucagon's effect on glucose's homeostasis.

The key assumption here was that GPROD was modulated by glucose and glucagon levels independently (Eq. 2). Insulin is a major regulator of glucagon secretion which in turn affects GPROD, but this action of insulin was not explicitly incorporated into the model, but rather was implicit and covered by the glucose and glucagon effects. At steady state (as the initial condition), glucose and glucagon levels (G_{ss} and GN_{ss}) were constant and, therefore, GPROD was constant (homeostasis). With perturbations, increased glucose levels reduced GPROD, while increased glucagon levels increased GPROD (Eq. 2). Furthermore, the ability of glucagon to increase GPROD was attenuated by MK-3577 exposure through an inhibitory E_{max} model ($I_{max,MK}$ and $IC_{50,MK}$) (Eq. 2).

$$GPROD = 0.5 \times GPROD_0 \times \left(\left(\frac{GE}{G_{ss}} \right)^{GPRG1} + \left(\left(1 - \frac{I_{max,MK} \times C_{MK}}{IC_{50,MK} + C_{MK}} \right) \left(\frac{GN_{central}}{GN_{ss}} \right)^{GPRG3} \right) \right) \quad (2)$$

where GPROD₀ is the steady-state glucose production rate, GE is glucose concentration in the effect compartment,

GPRG1 is a negative exponent for glucose's negative feedback on its own production, C_{MK} is the MK-3577 concentration in the central compartment, $GN_{central}$ is the glucagon concentration in the central compartment, and GPRG3 is a positive exponent for glucagon's stimulatory effect on glucose production.

GPROD₀ is defined in Eq. 3:

$$GPROD_0 = G_{ss} \times (CL_G + CL_{GI} \times I_{ss}) \quad (3)$$

where CL_G is the insulin-independent clearance of glucose, $CL_{GI} \times I_{ss}$ is the insulin-dependent clearance of glucose, and I_{ss} the steady-state insulin concentration.

The rate of change of glucose amount in the central compartment is shown in Eq. 4:

$$\frac{dA(6)}{dt} = GPROD + k_{PG} \times A(7) - (k_{GP} + k_G + k_{GI} \times C_I) \times A(6) \quad (4)$$

where $A(6)$ and $A(7)$ are glucose amounts in the central and peripheral compartments, respectively, k_{PG} and k_{GP} are the distributional rate constants between the central and peripheral compartments for glucose, and k_G and $k_{GI} \times C_I$ are the rate constants associated with the insulin-independent and insulin-dependent clearances of glucose, respectively. For the insulin-dependent clearance pathway, the higher the insulin concentration, C_I , the greater the clearance is for this pathway.

The rate of change of insulin amount in the central compartment, $A(5)$, is shown in Eq. 5:

$$\frac{dA(5)}{dt} = I_{ss} \times CL_I \times \left(\frac{C_{GC}}{G_{ss}} \right)^{IPRG} \times \left(1 - \frac{C_s}{IC_{50,S2} + C_s} \right) - k_I \times A(5) \quad (5)$$

where C_{GC} is the glucose concentration in the central compartment, IPRG is a positive exponent for glucose's stimulatory effect on insulin secretion, C_s is the Sandostatin concentration in the central compartment, $IC_{50,S2}$ is the Sandostatin concentration producing 50% of maximal inhibition on insulin secretion, and k_I is the elimination rate constant of insulin. The product of $I_{ss} \times CL_I$ equals to the steady-state insulin secretion rate. In this study, Sandostatin concentrations were not measured. Published literature (18,19) and product label for Sandostatin pharmacokinetics were used in the model.

The rate of change of glucagon amount in the central compartment, $A(4)$, is shown in Eq. 6:

$$\frac{dA(4)}{dt} = GN_{ss} \times CL_{GN} \times \left(1 + \frac{E_{max,MK} \times C_{MK}}{EC_{50,MK} + C_{MK}} \right) \times \left(1 - \frac{C_s}{IC_{50,S1} + C_s} \right) - k_{GN} \times A(4) \quad (6)$$

where CL_{GN} is the clearance of glucagon, $E_{max,MK}$ is the maximal effect of MK-3577 on stimulating glucagon secretion, $EC_{50,MK}$ is the MK-3577 concentration producing 50% of this maximal effect, $IC_{50,S1}$ is the Sandostatin concentration producing 50% of maximal inhibition on glucagon secretion, and k_{GN} is the elimination rate constant of glucagon. The product of $GN_{ss} \times CL_{GN}$ equals to the steady-state glucagon

Table II. Study Design (Glucagon Challenge in Healthy Subjects)

Study ^a (N=36)	MK-3577 single oral dose (mg)	MK-3577 dose clock time	Infusion ^b start time post MK-3577 dose (h)	Infusion ^b start clock time
Part 2 (N=12)	0 (placebo)	8 am	3	11 am
	100	8 am	3	11 am
	300	8 am	3	11 am
	900	8 am	3	11 am
Part 3 (N=12)	0 (placebo)	8 pm	12	8 am
	10	8 pm	12	8 am
	30	8 pm	12	8 am
	100	8 pm	12	8 am
	600	8 pm	12	8 am
Part 4 (N=12)	0 (placebo)	8 pm	12	8 am
	1	8 pm	12	8 am
	3	8 pm	12	8 am
	20	8 am	24	8 am
	40	8 am	24	8 am

^a Crossover with a balanced incomplete block design

^b Glucagon, Sandostatin, and insulin were infused for 2 h. Sandostatin was given to inhibit endogenous glucagon and insulin secretions. Insulin was given to provide a low basal level of insulin to reduce the degree of excessive glycemia

secretion rate. A stimulatory $E_{\max}$ component was added to glucagon secretion because an increase in glucagon concentrations was observed due to drug administration before the glucagon infusion began.

The concentrations for glucagon and glucose were log-transformed, while those for insulin were not because insulin concentrations in our study were within a much narrower range than glucose and glucagon concentrations.

Exponential inter-individual variability (IIV) models were used. An additive error model was used to describe the residual errors. Because the concentrations for glucagon and glucose were log-transformed, an additive residual error model on log-transformed data assumed a proportional error on the original scale. For insulin, a combined proportional and additive residual error model on non-log-transformed data was investigated and the proportional component was found to be near zero, so an additive residual error model was used for insulin as well.

CTS Method for T2DM Patients in the Phase IIa Study

Prior to this phase IIa study, all the data available for this compound came from healthy subjects. To project the MK-3577 effect on glycemic control in T2DM patients, the model for healthy subjects was adopted (including typical values, IIV, and residual error) with modifications to account for the differences between the two populations (details are shown below) and also to adjust for study design differences. The internal data from a lead compound within the same class were utilized to aid the modifications. The model structure for T2DM patients without glucagon challenge is shown in Fig. 1b.

The pharmacokinetic parameters for MK-3577 were kept the same between healthy and T2DM subjects because the lead compound showed no significant differences in pharmacokinetics between the two populations and there were no other reasons known to predict differences.

The model assumed that self-negative regulation of glucose production in T2DM patients was completely compromised (*i.e.*, $GPRG1=0$). This assumption is not completely physiological and T2DM patients do have some residual feedback regulation on glucose production by glucose. However, this residual feedback loop was inestimable using the lead compound data. Therefore, it was omitted from the model for T2DM. Similar finding and approach were also discussed in Silber's paper (9).

The insulin-independent clearance/uptake of glucose was assumed to be preserved (*i.e.*, the same as in healthy subjects). The CL_{GI} value for the insulin-dependent clearance of glucose in T2DM patients was fixed at 11% of that in healthy subjects based on the lead compound data.

In T2DM patients, the baseline (*i.e.*, steady-state) glucose level is much elevated compared to that in healthy subjects, accompanied with hyperinsulinemia and hyperglucagonemia (6,7). Mathematical derivations were done to explore whether the baseline conditions for glucose, insulin, and glucagon in T2DM patients are inter-related.

The compartment initialization was done by substituting healthy subject steady-state concentrations with those from T2DM patient (G_{SSP} , I_{SSP} , and GN_{SSP}) without modification of the volumes of distribution.

To derive G_{SSP} , I_{SSP} , and GN_{SSP} values, G_{SS} , I_{SS} , and GN_{SS} in the equations for healthy subjects (Eqs. 2–5) were kept the same in those for T2DM patients. For concentrations that were not G_{SS} , I_{SS} , or GN_{SS} (such as C_{GC} , C_I , and $GN_{central}$), G_{SSP} , I_{SSP} , and GN_{SSP} were used as substitute, respectively. The substitution also applied to the amount terms, such as $A(5)$ and $A(6)$ which became $I_{SSP} \times V_I$ and $G_{SSP} \times V_{GC}$, respectively. G_{SS} , I_{SS} , and GN_{SS} can be thought as the intrinsic set points for glucose, insulin, and glucagon in normal homeostasis, while G_{SSP} , I_{SSP} , and GN_{SSP} are the new set points that T2DM patients developed to cope with this disease condition.

Specifically for glucose, define $\frac{G_{SSP}}{G_{SS}} = 1 + \theta$ (Eq. 7), where θ is the fractional/fold increase in steady-state glucose concentration in T2DM compared to healthy subjects.

Fig. 1. Model schematics of the drug effect on glucagon and the interaction between glucagon, glucose, and insulin in healthy subjects during glucagon challenge (**a**) and in T2DM patients without glucagon challenge (**b**). *Solid lines* signify mass transfer, while *dashed lines* are for regulatory pathways but no mass transfer. CL_G insulin-independent clearance of glucose, CL_{GI} insulin-dependent clearance of glucose, GC glucose central compartment, GN glucagon, GP glucose peripheral compartment, $GPROD$ glucose production rate, I insulin, R_0 zero-order IV infusion, SN Sandostatin

For insulin, set Eq. 5 is equal to zero at time 0 and also set $C_S=0$ since no Sandostatin is in the body, then Eq. 5 turns into

$$I_{ss} \times CL_I \times \left(\frac{C_{GC}}{G_{ss}} \right)^{IPRG} = k_I \times A(5) \quad (7)$$

Then substitute C_{GC} with G_{SSP} , $A(5)$ with $I_{SSP} \times V_I$, and $k_I \times V_I$ with CL_I , then after rearrangement,

$$I_{SSP} = I_{ss} \times (1 + \theta)^{IPRG} \quad (8)$$

For glucagon at time 0, start with Eq. 2, set $GPRG1=0$ because of no self-regulation of glucose on GPROD in T2DM patients, set $C_{MK}=0$ since no MK-3577 is in the body at time 0, and substitute $GN_{central}$ with GN_{SSP} , then Eq. 2 turns into

$$GPROD_{0P} = 0.5 \times GPROD_0 \times \left(1 + \left(\frac{GN_{SSP}}{GN_{ss}} \right)^{GPRG3} \right) \quad (9)$$

where $GPROD_0$ is for healthy subjects and $GPROD_{0P}$ is for T2DM patients.

Then, set Eq. 4 for glucose equal to zero at time 0, substitute $GPROD_0$ with $GPROD_{0P}$, set the rates between the central and peripheral compartments to be the same (*i.e.*, $k_{PG} \times A(7) = k_{GP} \times A(6)$), substitute $A(6)$ with $G_{SSP} \times V_{GC}$, and C_I with I_{SSP} , then Eq. 4 turns into

$$GPROD_{0P} - (k_G + k_{GI} \times I_{SSP}) \times G_{SSP} \times V_{GC} = 0$$

Substitute $GPROD_{0P}$ with the right side of Eq. 9, and after rearrangement,

$$GN_{SSP} = \left(\frac{2 \times (1 + \theta) \times (CL_G + CL_{GI} \times I_{SSP})}{CL_G + CL_{GI} \times I_{SSP}} - 1 \right)^{\frac{1}{GPRG3}} \times GN_{ss} \quad (10)$$

With Eqs. 7, 8, and 10, if the θ value was estimated using the ratio of G_{SSP} and G_{ss} , then I_{SSP} and GN_{SSP} values could be calculated if I_{ss} , GN_{ss} , and other parameters were available either by assuming they were the same between healthy and patients or making adjustment such as for CL_{GI} .

Once compartment initialization equations were derived, the same set of equations for healthy subjects (Eqs. 2–6) was used in T2DM patients, except that for Eq. 6 GN_{SSP} was used in place of GN_{ss} .

An exponential IIV model was used for θ . The typical value of θ for the population was fixed at 1. This twofold increase in baseline FPG in T2DM vs. healthy subjects was based on four internal studies in T2DM patients after applying the same inclusion criteria of baseline FPG being ≥ 140 and ≤ 240 mg/dL as the current phase IIa study. The actual baseline FPG in the current study was unavailable prior to the interim analysis due to blinding. The IIV was fixed at 51% coefficient of variation (CV) based on the lead compound data.

Because the glucagon challenge and sampling time points took place under fasting condition, the model did not have any meal component, and FPG was the pharmacodynamic output

from the model. However, 24-h WMG was the pharmacodynamic endpoint for the phase IIa study. Therefore, a linear model correlating FPG and WMG was developed using the data from the Diabetes Control and Complications Trial (DCCT). The DCCT was a clinical study conducted in 1,441 type 1 diabetic patients treated with insulin.

A total of 1,000 trials, which is routinely done for CTS, with various MK-3577 doses (QD and BID, am and pm) in each trial and 82 patients in each dose cohort were simulated. Eighty-two was the maximal sample size per dose cohort for the phase IIa study. IIV and residual error were included in CTS, but parameter uncertainty was not. Including parameter uncertainty is valuable if actual data for parameter estimation are lacking and can only be guessed (*e.g.*, for a hypothetical drug) or when a great deal of uncertainty is involved (20). Given that we had prior knowledge from the lead compound within the same class, including parameter uncertainty may not add much insight.

To estimate the mean reduction in 24-h WMG, the 80% prediction interval of the mean reduction from baseline was used. Specifically, all subjects' WMG changes from baseline within each dose cohort were ranked; then the 10–90th percentiles would give the 80% prediction interval. If this prediction interval was entirely below or above the cutoffs (such as 30 mg/dL), then the dose is increased or decreased.

Once the observed data from the interim analysis became available, the simulated results were compared to the observed data. Additional simulations were done for a typical subject (*i.e.*, without IIV and residual error) with the baseline FPG value set to the observed mean value.

Software

NONMEM version 7.2 (ADVAN13, importance sampling) and R version 3.0.1 were used for modeling and NONMEM version 7.2 and SAS version 9.1 were used for CTS. Berkeley Madonna version 9.0 was used for additional simulations after the observed data from the interim analysis became available.

RESULTS

Modeling Method for MK-3577 Population Pharmacokinetics

Visual predictive check of MK-3577 concentration profiles in healthy subjects in selected dose cohorts are shown in Fig. 2. The model was able to capture MK-3577 pharmacokinetic profiles. The bioavailability of MK-3577 increased by 21% for pm dosing compared to am dosing. The elimination of MK-3577 was found to follow a circadian rhythm which became more apparent when the samples were taken more than 24 h, such as during part 4 of the study. k_{min} and k_{max} were estimated to be 0.0403 and 0.263 (1/h), respectively, with 19.4% CV as IIV. T_{KM} was estimated to be 11.7 h with 19.5% CV.

Modeling for Healthy Subjects with Glucagon Challenge

Visual predictive check of glucose, glucagon, and insulin pre- and postchallenge in healthy subjects are shown in Fig. 2. Model parameters are shown in Table III.

In general, the model was able to capture the concentration profiles very well for glucose, glucagon, and insulin as demonstrated by the concordance of the prediction intervals from the visual predictive check with the observed values. As shown in Fig. 2 (panel, dose=0 mg_PART=2), when there was no drug in the body, the glucagon infusion caused an influx of glucose in plasma as expected, and the glucose concentration was doubled under this experimental condition. When there was drug in the body (Fig. 2; panels, dose=3 mg_PART=4, dose=30 mg_PART=3, and dose=300 mg_PART=2), the influx of glucose decreased in a dose-dependent manner with $I_{\max}$ estimated to be 0.961 and IC_{50} to be 13.9 nM. A single 300 mg dose of MK-3577 was able to completely block the effect of glucagon on glucose

and even caused the glucose level to decrease lower than the baseline value, and the model was able to capture this trend.

The glucagon concentration increased during glucagon infusion, so did the insulin concentration due to the basal insulin infusion. The interesting observation was that prior to the glucagon infusion, the glucagon concentration already increased when a large MK-3577 dose (e.g., 300 mg) was given and correspondingly the insulin concentration decreased prechallenge (Fig. 2; panel, dose=300 mg_PART=2, 0–3 h). The hypothesis for the increase in glucagon concentration prechallenge was that the body sensed a blockage of glucagon effect when the drug was present and hence the glucagon secretion was increased *via* a compensatory feedback loop. This hypothesis was supported by the finding that

Fig. 2. Visual predictive check of MK-3577 concentrations (a), glucose (b), glucagon (c), and insulin (d) pre- and postchallenge in healthy subjects in selected dose cohorts. Black dots are for observed individual values. Eight hundred datasets were simulated. The black lines show the median, 10th, and 90th percentiles of the simulated individual predictions. Time 0 was when MK-3577 dose was given

Table III. Model Parameters for Glucose, Glucagon, and Insulin in Healthy Subjects

Parameter (unit)	Estimate (%RSE)	IIV (%CV)	Description
Glucose			
G_{SS} (mg/dL)	91.9 (1.3)	6.1 <i>FIX</i>	Glucose SS concentration
CL_G (dL/kg/h) ^a	0.463 (36)	–	Glucose insulin-independent clearance
CL_{GI} (dL/kg/h/ μ U/mL) ^a	0.102 (30)	–	Glucose insulin-dependent clearance
Q_G (dL/kg/h) ^a	0.180 (14)	–	Glucose intercompartmental clearance
V_{GC} (dL/kg) ^a	0.845 (23)	28.8 <i>FIX</i>	Glucose central compartment volume
V_{GP} (dL/kg) ^a	0.301 (15)	–	Glucose peripheral compartment volume
K_{GE} (1/h)	0.084 (43)	–	Glucose k_{eo} for glucose regulation
$I_{max,MK}$	0.961 (1.7)	–	I_{max} of MK-3577 inhibitory effect on glucagon stimulation on glucose production
$IC_{50,MK}$ (nM)	13.9 (14)	77.7	IC_{50} of MK-3577 inhibitory effect on glucagon stimulation on glucose production
GPRG1	–2.08 (26)	–	Glucose negative feedback on glucose production
GPRG3	4.05 (10)	–	Glucagon stimulatory effect on glucose production
RESG (%)	7.54 (8.1)	–	Glucose residual %CV
Insulin			
I_{SS} (μ U/mL)	4.14 (4.8)	33.3 <i>FIX</i>	Insulin SS concentration
CL_I (L/kg/h)	1.40 (13)	26.3 <i>FIX</i>	Insulin clearance
V_I (L/kg)	0.320 (33)	–	Insulin volume
IPRG	2.30 (13)	–	Glucose stimulatory effect on insulin secretion
$IC_{50,S2}$ (ng/mL)	0.921 (23)	–	Sandostatin IC_{50} on insulin secretion
RESI (μ U/mL)	1.38 (7.4)	–	Insulin residual SD
Glucagon			
GN_{SS} (pg/mL)	58.3 (3.3)	10.6 <i>FIX</i>	Glucagon SS concentration
CL_{GN} (L/kg/h)	3.19 (3.4)	18.4 <i>FIX</i>	Glucagon clearance
V_{GN} (L/kg)	1.39 (7.7)	–	Glucagon volume
$E_{max,MK}$	0.788 <i>FIX</i>	–	E_{max} of MK-3577 stimulatory effect on glucagon secretion
$EC_{50,MK}$ (nM)	575 <i>FIX</i>	–	EC_{50} of MK-3577 stimulatory effect on glucagon secretion
$IC_{50,S1}$ (ng/mL)	5.50 (10)	–	Sandostatin IC_{50} on glucagon secretion
RESGN (%)	30.3 (5.0)	–	Glucagon residual %CV

Some parameter estimates were fixed to values obtained from initial runs (*e.g.*, $E_{max,MK}$ and $EC_{50,MK}$), or to values from the lead compound within the same class to aid model minimization

RSE relative standard error, IIV inter-individual variability, SS steady state

^a Parameter estimates should be interpreted with caution. Because a labeled glucose dose was not given, the glucose V and CL terms (which contain V components) actually could not be estimated explicitly. All could be estimated were the ratios of CL to V terms. Another model with different parameterization which estimated the ratio of V_{GP}/V_{GC} , K_G , K_{GI} , and K_{GP} was explored. Despite different parameterization, both models showed very similar fits for glucose, glucagon, and insulin. Therefore, the model estimates shown here were used as the final estimates for simulations

chronic administration of glucagon receptor antagonists could lead to α cell hypertrophy or hyperplasia which resulted in hypersecretion of glucagon (21). Therefore, a stimulatory effect of MK-3577 on glucagon secretion was added, and E_{max} was estimated to be 0.788 and EC_{50} 575 nM. The EC_{50} value was approximately 42-fold larger than the IC_{50} value indicating that the drug was not a potent secretagogue on glucagon.

Even though the glucagon concentration increased in the circulation, the glucagon effect on glucose production was still blocked by the drug, thus the glucose concentration decreased prechallenge, which drove the insulin secretion to decrease which then in turn resulted in a decrease in the insulin concentration prior to the challenge. The model was able to describe this phenomenon.

The insulin-independent and insulin-dependent clearances of glucose were estimated to be comparable to each other (0.463 *vs.* 0.422 dL/kg/h, where $0.422 = CL_{GI} \times I_{ss} = 0.102 \times 4.14$ dL/h). The exponent for the glucagon stimulatory effect on glucose production was almost doubled compared to that for the glucose negative feedback effect on its own production (*i.e.*, GPRG3=4.05 *vs.* GPRG1=–2.08),

suggesting that glucagon is more potent in increasing glucose production than glucose's counter mechanism to reduce its own production.

A one-compartment model was used for Sandostatin pharmacokinetics (0.121 L/kg/h and 0.194 L/kg as clearance and volume of distribution, respectively). The published data indicate a two-compartmental behavior for Sandostatin, but detailed model estimates were unavailable. The simplification is not expected to cause significant deviation because the distribution phase of Sandostatin is rapid compared to the elimination phase (distribution and elimination $t_{1/2}$'s are 0.2 and 1.7 h, respectively). Sandostatin inhibited the endogenous secretions of insulin and glucagon, and the inhibitory effect was more potent towards insulin ($IC_{50,S2}$ =0.921 ng/mL) than towards glucagon ($IC_{50,S1}$ =5.50 ng/mL). The I_{max} was fixed to one for both inhibitions.

The goodness-of-fit plots are shown in Fig. 3. The model well-predicted glucose concentrations. For glucagon, the model somewhat underpredicted at high concentrations and overpredicted at lower concentrations. For insulin, the model

Fig. 3. Goodness-of-fit plots for the NONMEM model in healthy subjects

fit was adequate. The lack of fit for glucagon was likely due to model misspecification and the inability of the study design (*e.g.*, limited sampling times) to allow for a more complex model. Because glucose was our primary focus, this model was deemed to be fit for purpose.

CTS for the Phase IIa Study with T2DM Patients

Figure 4 shows the goodness-of-fit plots for the linear model correlating the FPG and WMG values. As shown in Fig. 4, the model was able to predict the 24-h WMG value very well using four glucose concentrations before three meals and at the bedtime. The prebreakfast measurement was strictly at a fasting condition, while the remaining three measurements were not. Despite this, our internal data have

shown that the prelunch, presupper, and bedtime glucose measurements were very similar to that prebreakfast.

Table IV shows the parameter estimates for this linear model. All the slopes (a , b , c , and d) were found to be significantly different from zero. The magnitudes of these slopes were quite similar, with prelunch measurements contributing 30% less to the WMG value compared to the presupper measurements.

Once the parameter estimates were obtained for the linear model, the NONMEM model simulated output (*i.e.*, FPG values) before three meals and at the bedtime were put into the linear model to project the WMG values. When the drug was in the body, the four FPG values would be different because the drug concentrations would be different resulting in different glycemic effects at these time points.

Fig. 4. Goodness-of-fit plots for the linear model for WMG. The *solid line* is line of unity, and the *curve* is loess smoothed curve

The simulation results for WMG decrease from baseline and the probability (%) of dose adjustment are shown in Table V. According to CTS and with the bounds of 30–40 or 60 mg/dL WMG reduction for QD or BID doses, respectively, the current doses (10 mg QD am, 6 mg QD pm, and 25 mg BID) were near optimal. The am dose would likely be best adjusted to either 7 or 8 mg so that the chance of dose reduction required would decrease to less than 3%. The pm dose would likely be best adjusted to 5 mg so that the chance of no dose adjustment required would be as high as 96%. The BID dose would not require any increase. Because the required dose adjustment was small, additional drug material production was not initiated.

Additional Simulations After Observed Data Became Available

Once the observed data from the interim analysis for the phase IIa study became available (22), the simulation results were compared to the observed. The comparison showed that simulations overpredicted the WMG lowering effect for MK-3577. One possible explanation for this overprediction was that the observed mean baseline FPG value (150 mg/dL) was much lower than that used in the simulations (184 mg/dL). Internal data have shown that the higher the baseline glucose levels were, the greater the glucose lowering effects would be for antidiabetic drugs.

To test this hypothesis, additional simulations were done for a typical T2DM patient with a baseline FPG value of 150 mg/dL. The observed and simulated WMG and FPG results are shown in Table VI. The simulation results were very comparable to those observed once the typical baseline FPG value was set to the mean observed value. For comparison purposes, the observed changes from baseline *vs.* placebo were used. The placebo values reflect the effects of diet and behavioral changes and disease progression if any during the study duration. Since these effects were not included in the model, the model predictions were, in essence, placebo-adjusted values.

DISCUSSION

In this work, we have presented a semi-mechanistic model to describe the effect of a glucagon receptor antagonist on glucagon and the interaction between glucagon, insulin, and glucose in healthy subjects who received glucagon challenge and how to adapt the model for the T2DM patient population and how to correlate FPG and WMG values to aid dose adjustment for the interim analysis of a phase IIa study through simulations.

Based on the pharmacokinetic/pharmacodynamic profile of MK-3577 derived from the FIH study, it was anticipated that 10 mg QD am would have a similar 24-h mean functional blockade of the glucagon receptor as 6 mg QD pm (*i.e.*, ~50%), while providing reduced levels of blockade during the overnight and daytime periods, respectively. In contrast, 25 mg BID will provide sustained functional blockade of the glucagon receptor (~86%) throughout the day. The observed data from the phase IIa study confirmed that that 25 mg BID produced robust glycemic efficacy compared to 10 mg QD am and 6 mg QD pm (22), demonstrating that sustained 24-h blockade of the glucagon effect would be necessary to achieve clinically important glucose-lowering efficacy, compared to transient blockade.

Since A1c reflects long-term (~12 week) glycemic control, it was not used as a key endpoint in the phase IIa study due to the short duration of the treatment period (4 weeks). In order to provide an endpoint that reflected overall control and could be obtained within a shorter treatment period, a 24-h WMG was used. This measure was derived from multiple glucose values collected during both fasting and postmeal periods. Based on an analysis of daily mean glucose (an endpoint generally similar to WMG), a change of approximately 30 to 35 mg/dL will correlate with a 1% change in A1C (23).

The data from the DCCT were used to build a correlation model between WMG and FPG (Table IV). The same model structure to correlate WMG to FPG was also used to fit the data from the lead compound and yielded similar parameter estimates (results not shown). The fact that

Table IV. Parameter Estimates for the Linear Model Between FPG and WMG

Solution for fixed effects					
Effect	Estimate	Standard error	df	t value	Pr > t
Intercept	22.0	0.461	26,139	47.6	<0.0001
Prebreakfast	0.262	0.0018	26,139	144	<0.0001
Prelunch	0.190	0.0017	26,139	111	<0.0001
Presupper	0.271	0.0016	26,139	167	<0.0001
Bedtime	0.238	0.0016	26,139	151	<0.0001
SD (η_{AN})	9.28				
SD (ε)	22.4				

WMG = $a \times [\text{prebreakfast}] + b \times [\text{prelunch}] + c \times [\text{presupper}] + d \times [\text{bedtime}] + \text{intercept} + \eta_{AN} + \varepsilon$, where η_{AN} is inter-individual variability and ε is the residual error. All glucose concentrations were in milligrams per deciliter

the two independent datasets with different mechanisms and treatments in two different patient populations provided similar estimates gave us significant confidence in using the linear model.

The sum of the slope (a , b , c , and d) estimates from the linear model was almost 1 (0.961), indicating that the intercept estimate (22.0 mg/dL) was mainly coming from the contribution of meals. Since the intercept was a

constant, this linear model can be applied to antidiabetic drugs that predominantly affect FPG, but less affect postprandial glucose excursion. Using this linear equation for dipeptidyl peptidase-4 (DPP4) inhibitors, for instance, would presumably underestimate the WMG-lowering effect.

When the observed data from the interim analysis for the phase IIa study became available, the comparison between the simulated vs. observed data indicated that simulations overpredicted the WMG-lowering effect for MK-3577. At that time, one could easily conclude that the model or assumptions utilized by the model was not appropriate. However, with further interrogation, we were able to identify one plausible explanation for this overprediction: the observed mean baseline FPG value was much lower than that used in CTS. With the appropriate baseline glucose levels, the projected glucose-lowering effects were comparable to those observed. To avoid this type of discrepancy proactively, it will be useful for the modelers to have early access to important baseline values and this can be done in a blinded fashion if the study is still blinded.

Table V. Simulation Results for WMG Decrease from Baseline and Probability of Dose Adjustment

Dose time	MK-3577 dose (mg)	Simulated LS-mean WMG decrease (mg/dL)	Probability (%) of dose change ^a		
			Decrease	Keep	Increase
QD, am	5	27.2	0	68.8	31.2
	6	30.3	0	89.1	10.9
	7	33.1	0.9	96	3.1
	8	35.0	2.6	96.9	0.5
	9	37.1	8.4	91.6	0
	10	39.4	20.5	79.5	0
	11	40.8	30.9	69.1	0
	12	42.4	44.2	55.8	0
	14	45.2	66.7	33.3	0
	3	24.8	0	44.3	55.7
	4	29.3	0.1	82.7	17.2
	5	33.8	1.4	96.3	2.3
	6	37.0	8	91.6	0.4
	7	40.2	25.4	74.6	0
	8	43.0	49.5	50.5	0
QD, pm	9	45.4	67.8	32.2	0
	10	47.6	83.9	16.1	0
	16	76.6	—	100	0
	18	78.7	—	100	0
	20	80.3	—	100	0
	22	81.7	—	100	0
	25	83.7	—	100	0
	30	85.8	—	100	0
BID					

The doses prior to the phase IIa interim analysis are shown in italic LS least square

^a For QD doses, the dose change rules were that if the 80% prediction interval of the study mean reduction in WMG was <30 mg/dL, increase the dose and if it was >40 mg/dL, decrease the dose. For BID, there was only an increase rule if the 80% prediction interval was <60 mg/dL

Table VI. Observed and Simulated WMG and FPG Change from Baseline vs. Placebo at Week 4

Dose regimen	Observed (difference in LS means) ^a	Simulated (typical value) ^b
WMG change from baseline vs. placebo at week 4 (mg/dL)		
10 mg QAM	-17.2	-24.5
6 mg QPM	-23.4	-21.2
25 mg BID	Unavailable ^c	-47.9
FPG change from baseline vs. placebo at week 4 (mg/dL)		
10 mg QAM	-11.5	-5.84
6 mg QPM	-21.8	-28.3
25 mg BID	-36.0	-43.5

^a Based on a longitudinal data analysis model including terms for treatment, period, the interaction of treatment by period, subject effect, etc

^b For simulations, 150 mg/dL as baseline FPG was assumed. Therefore, the baseline WMG was calculated as 166.13 mg/dL using the linear correlation equation

^c Patients on 25 mg BID did not undergo 24-h glucose sampling due to study discontinuation

Overall, we were able to leverage knowledge learned from the lead compound and internal expertise in glucagon receptor pathway and from the literature regarding glucose and insulin regulation to build a semi-mechanistic model to describe the interaction between key factors in glycemic control. External validation using new data would provide more robust assessment of the model and the validity of underlying assumptions. Additionally, including a meal component in the model would allow a more mechanistic prediction of WMG.

CONCLUSION

A semi-mechanistic model was developed to capture the effect of a glucagon receptor antagonist on glucagon and the interaction between glucose, glucagon, and insulin. A linear model to correlate FPG to WMG was developed and provided robust predictions to assist with the dose adjustment for a phase IIa study. The simulation results demonstrated the importance of early access to key baseline characteristics in accurately predicting outcome.

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