Target-Mediated Pharmacokinetic and Pharmacodynamic Model of Exendin-4 in Rats, Monkeys, and Humans

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ABSTRACT:
A mechanism-based pharmacokinetic-pharmacodynamic (PK/PD) model was developed for exendin-4 to account for receptor-mediated endocytosis via glucagon-like peptide 1 receptor (GLP-1R) as the primary mechanism for its nonlinear disposition. Time profiles of exendin-4 concentrations after intravenous, subcutaneous, and continuous intravenous infusion doses in rats, intravenous and subcutaneous doses in monkeys, and intravenous infusion and subcutaneous doses in humans were examined. Mean data for glucose and insulin after glucose challenges during exendin-4 treatment in healthy rats were analyzed. The PK model components included receptor binding, subsequent internalization and degradation, nonspecific tissue distribution, and linear first-order elimination from plasma. The absorption rate constant (kₐ) decreased with increasing doses in all three species. The clearance from the central compartment (CLc) (rats, 3.62 ml/min; monkeys, 2.39 ml·min⁻¹·kg⁻¹; humans, 1.48 ml·min⁻¹·kg⁻¹) was similar to reported renal clearances. Selected PK parameters (CLc, Vₕ, and kₐ) correlated allometrically with body weight. The equilibrium dissociation constant (Kₑ) was within the reported range in rats (0.74 nM), whereas the value in monkeys (0.12 pM) was much lower than that in humans (1.38 nM). The effects of exendin-4 on the glucose-insulin system were described by a feedback model with a biphasic effect equation driven by free exendin-4 concentrations. Our generalized nonlinear PK/PD model for exendin-4 taking into account of drug binding to GLP-1R well described PK profiles after various routes of administration over a large range of doses in three species along with PD responses in healthy rats. The present model closely reflects underlying mechanisms of disposition and dynamics of exendin-4.

Introduction
Exendin-4 is a 39-amino acid glucagon-like peptide 1 (GLP-1) analog, sharing approximately 53% sequence identity with mammalian GLP-1 (Doyle and Egan, 2007). Exendin-4 binds to pancreatic GLP-1 receptors (GLP-1R) to exhibit antidiabetic actions, including glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, slowing of gastric emptying, satiety, and in preclinical models, protection of β-cells. In addition to the pancreas, GLP-1Rs are also expressed in various tissues such as brain, lung, and kidneys (Körner et al., 2007).

The disposition of exendin-4 in humans has been reported as linear over the therapeutic dose range from 5 to 10 μg (Cvetković and Plosker, 2007). However, the pharmacokinetics (PK) of exendin-4 was reported as nonlinear in monkeys (Ai et al., 2008). In addition, the Bateman function could not describe the concentration-time profiles over certain ranges of doses in rats with one set of elimination parameter values (Gedulin et al., 2008), suggesting that the PK in rats may also be nonlinear. Furthermore, the simple Bateman function does not well represent the underlying mechanism of exendin-4 disposition, such as receptor binding and internalization. The major route of exendin-4 elimination was suggested to be glomerular filtration with subsequent enzyme degradation (Copley et al., 2006). However, in rats with kidneys surgically removed, exendin-4 slowly disappeared from the system (Parkes et al., 2001a), indicating the existence of nontrenal clearance. Because GLP-1Rs exist in various tissues and after binding to exendin-4, exendin-4-GLP-1R complexes are internalized and targeted for further degradation, we reasoned that receptor-mediated endocytosis and degradation may be responsible for the nontrenal clearance and the observed nonlinear behavior of exendin-4.

A general model for drugs exhibiting target-mediated drug disposition (TMDD) exists (Mager and Jusko, 2001). After administration, drug can be distributed to the peripheral compartment, directly eliminated, or bind to receptors. The drug-receptor complexes can be eliminated or dissociated to free receptors. The TMDD model uses receptor binding and receptor-mediated endocytosis as the primary mechanism of nonlinear drug disposition. We successfully captured the disposition of exendin-4 in diabetic rats using the TMDD model (Gao and Jusko, 2011). However, the PK of exendin-4 in other species has not been assessed using mechanistic modeling.

In general, the in vivo pharmacological effects of exendin-4 have been evaluated either qualitatively or by comparing empirical measures such as the area under the plasma glucose and insulin concent-
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In another study, male SD rats (80–420 g b.wt., n = 4–8) were infused intravenously using the same volume of saline or exendin-4 at 3, 30, 300, and 3000 pmol·kg⁻¹·min⁻¹ for 2 h. At 30 min after beginning the infusion, d-glucose (5.7 mmol/kg) was injected intravenously at a rate of 0.5 ml/min over 2 to 3 min. Plasma glucose was determined by immobilized oxidase chemistry on a YSI 2300 Stat Plus (YSI Inc., Yellow Springs, OH), and insulin was determined by radioimmunoassay (Linco Research, St. Charles, MO).

In the monkey PK study (Ai et al., 2008), male rhesus monkeys (4.3 ± 0.7 kg b.wt., n = 3) were given either a single subcutaneous injection of 1, 3, or 10 µg/kg or a single intravenous injection of 3 µg/kg. Serum exendin-4 concentrations were measured using a radioimmunoassay. The linear range of this assay was 25 to 2000 pg/ml, and the limit of quantitation of was 25 pg/ml.

Data from three human studies were included in the current analysis. In study A (Kolterman et al., 2005), eight subjects (88.5 ± 9.4 kg b.wt.) received 0.1, 0.2, 0.3, or 0.4-µg/kg subcutaneous doses of exendin-4. In study B (Kolterman et al., 2005), eight subjects (88.8 ± 12.1 kg b.wt.) received single subcutaneous doses of 0.02, 0.05, or 0.1 µg/kg. In study C (Degn et al., 2004), 11 subjects (21–29 kg/m² body mass index) received an intravenous infusion of exendin-4 at 0.066 pmol·kg⁻¹·min⁻¹ (0.276 ng·kg⁻¹·min⁻¹) for 360 min. Plasma exendin-4 concentrations were measured by Amylin Pharmaceuticals, Inc. using an immunoenzymetric assay.

**Pharmacodynamic Model.** For initial data evaluation, mean profiles of exendin-4 for each intravenous dose obtained from rats were used to perform a noncompartamental analysis (NCA) and curve fitting to a biexponential equation (C = C1·e⁻λ₁·t + C2·e⁻λ₂·t) using WinNonlin 5.0 (Pharsight, Mountain View, CA) to evaluate dose-dependent changes in clearance (CL), steady-state volume of distribution (Vss), and distributional clearance (CLd).

For the next stage, a mechanism-based modeling approach was used for data analysis. The general scheme of the applied PK/PD model is presented in Fig. 1. The free exendin-4 (C) in plasma can bind to GLP-1R to form drug-receptor complex (RC), distribute to and from tissues (Aᵢ) by first-order rates (kᵢ and k₋ᵢ), and be directly eliminated (kₑ). The RC can dissociate at a first-order rate (kᵢ) and be internalized and degraded (kᵢ). The GLP-1R (R) is assumed to remain constant (Rₑ). The TMDD PK model can be described by eqs. 1 to 3:

\[ \frac{dC}{dt} = \text{input(t)} - (kᵢ + kₑ) \times C + kᵢ \times Aᵢ/Vᵢ - kᵢ \times R \times C + kᵢ \times RC; \]

\[ C(0) = \text{dose} / Vᵢ \text{ (iv) or 0 (sc, infusion.)} \] (1)

\[ \frac{dAᵢ}{dt} = kᵢ \times C \times Vᵢ - kᵢ \times Aᵢ; \ Aᵢ(0) = 0 \] (2)

\[ \frac{dRC}{dt} = kₑ \times (Rₑ - RC) \times C - (kᵢ \times kₑ) \times RC; \ RC(0) = 0 \] (3)

where \( Vᵢ \) represents the volume of the free exendin-4 (central) compartment.

**Materials and Methods**

The time profiles of exendin-4 concentrations in rats and humans were obtained from studies conducted by Amylin Pharmaceuticals, Inc. (San Diego, CA). The mean concentrations of exendin-4 in monkeys were captured by obtaining from studies conducted by Amylin Pharmaceuticals, Inc. (San Diego, CA). The mean concentrations of exendin-4 were obtained by radioimmunoassay (Linco Research, St. Charles, MO).

In the rat PK study, male Sprague-Dawley (SD) rats (350–370 g b.wt., n = 4–7) received exendin-4 via three different routes—intravenous, subcutaneous, or intravenous infusion—at three doses: 0.5, 5, and 50 nmol/h. Serum exendin-4 concentrations were measured using a two-site sandwich assay developed at Amylin Pharmaceuticals, Inc. using an immunoenzymetric assay.

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The input function for eq. 1 after subcutaneous doses is as follows:

\[ \text{input}(t) = k_a \times F \times \text{Dose} \times \exp(-k_t \times t)/V_C \]  

(4)

where \( k_a \) is the first-order absorption rate constant and \( F \) is the absolute bioavailability after subcutaneous doses.

**Pharmacodynamic Model.** The PD model proposed for insulinotropic effects of exendin-4 is shown in Fig. 1. The basic structure, the feedback model, represents the interregulated interaction between glucose and insulin: glucose (Glu) stimulates insulin secretion with a linear stimulation factor \( S_{\text{Glu}} \), and insulin (Ins) stimulates glucose uptake with a linear stimulation factor \( S_{\text{Ins}} \). The homeostasis of glucose and insulin was described by two indirect response models: \( k_{\text{outG}} \) and \( k_{\text{outI}} \) are the first-order output rate constants, and \( k_{\text{inG}} \) and \( k_{\text{inI}} \) are the zero-order input rate constants with the relationship of \( k_{\text{inG}} = k_{\text{outG}}/S_{\text{Glu}} \) and \( k_{\text{inI}} = k_{\text{outI}}/S_{\text{Ins}} \), where \( S_{\text{Glu}} \) and \( S_{\text{Ins}} \) are the basal glucose and insulin concentrations.

For initial evaluation, the simple feedback model was fitted to all the paired glucose and insulin profiles. All of the parameter estimates were comparable between dose groups, except for parameter \( S_{\text{Glu}} \). The next step was to apply the feedback model to glucose and insulin profiles for all rats, only allowing \( S_{\text{Glu}} \) to change among the dose groups.

For the next stage, a mechanism-based PK/PD modeling approach was used for data analysis. Drug concentrations were simulated using the TMDD PK model and parameter values from the PK study. The PK driving function enhances glucose-dependent stimulation of insulin secretion by the biphasic Adair function (\( S_b \)). Equations 5 to 7 show the feedback model for glucose and insulin concentrations:

\[
\frac{d\text{Glu}}{dt} = \frac{k_{\text{inG}} - k_{\text{outG}} \times (1 + S_{\text{Glu}} \times (\text{Ins} - I_0))}{V_i} \times \text{Glu}; \quad \text{Glu}(0) = \text{Dose}/V_i + G_b \]  

(5)

\[
\frac{d\text{Ins}}{dt} = \frac{k_{\text{inI}} \times (1 + S_{\text{Ins}} \times ((\text{Glu} - G_b) \times (1+ S_0)) - k_{\text{outI}} \times \text{Ins};} \quad \text{Ins}(0) = I_0 \]  

(6)

\[
S_z = \frac{S_{\text{max}} \times C}{k_1 + C + k_2 \times C^2} \]  

(7)

where \( S_{\text{max}} \) is the maximal stimulation factor of the response and \( k_1 \) and \( k_2 \) are constants in the Adair function. The baseline conditions \( G_0 \) and \( I_0 \) were fixed as the measured pre-dose values.

Using RC (eq. 8) as the driving function for the effects of exendin-4 was also tested:

\[
S_{\text{A,RC}} = \frac{S_{\text{max,RC}} \times \text{RC}}{k_1 \times \text{RC} + k_2 \times \text{RC}^2} \]  

(8)

All computer fittings and simulations were performed using ADAPT II (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA) with the maximal likelihood method. The variance was simulated with the Student's t-test. The model was described as the 95% confidence interval (CI) and \( F \) value.

### Results

**Pharmacokinetics.** The TMDD model has been used to capture exendin-4 disposition in diabetic rats (Gao and Jusko, 2011) and adequately described exendin-4 PK in rats, monkeys, and humans in this report. As shown in the model scheme (Fig. 1), the TMDD model consists of target-binding (\( k_{\text{on}} / k_{\text{off}} \)), internalization and degradation of the receptor complex (\( k_{\text{int}} \)), nonspecific tissue distribution (\( A_T \)), and a linear elimination pathways (\( k_d \)) from the drug.

**Rat PK.** The mean exendin-4 concentration-time profiles after various doses in rats are shown in Fig. 2. The PK profiles show biexponential decline with typical characteristics of TMDD where low doses showed rapid decline in early times after intravenous injection and after stopping the intravenous infusion. After intravenous injection, terminal half-lives ranged from 20 to 40 min with

![Graph showing exendin-4 concentration versus time profiles](image_url)

**Fig. 3.** Exendin-4 concentration versus time profiles after single intravenous (iv) and s.c. doses of 1, 3, and 10 \( \mu \)g/kg in monkeys. Symbols are mean drug concentrations with error bars representing S.E. (n = 3), and solid lines are fitted profiles.

### Table 1

Parameters obtained from NCA analysis of concentration-time profiles in rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>( \lambda_1 )</th>
<th>( \lambda_2 )</th>
<th>CL</th>
<th>( V_i )</th>
<th>( CL_{D} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.297</td>
<td>0.0363</td>
<td>4.99</td>
<td>44.8</td>
<td>1.08</td>
</tr>
<tr>
<td>5</td>
<td>0.133</td>
<td>0.0240</td>
<td>3.40</td>
<td>57.3</td>
<td>0.751</td>
</tr>
<tr>
<td>50</td>
<td>0.137</td>
<td>0.0174</td>
<td>3.39</td>
<td>68.2</td>
<td>0.818</td>
</tr>
</tbody>
</table>

### Table 2

Parameter estimates obtained from the time profiles of exendin-4 in rats with the TMDD model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{\text{int}} ) min(^{-1} )</td>
<td>Elimination rate constant</td>
<td>0.0839 (10)</td>
</tr>
<tr>
<td>( k_{\text{inG}} ) min(^{-1} )</td>
<td>Intercompartmental rate constant</td>
<td>0.0282 (15)</td>
</tr>
<tr>
<td>( k_{\text{inI}} ) min(^{-1} )</td>
<td>Intercompartmental rate constant</td>
<td>0.0213 (5)</td>
</tr>
<tr>
<td>( V_i ) ml</td>
<td>Central volume of distribution</td>
<td>43.2 (12)</td>
</tr>
<tr>
<td>( k_{\text{inG}} ) nM(^{-1} ) min(^{-1} )</td>
<td>Second-order binding constant</td>
<td>0.0207 (42)</td>
</tr>
<tr>
<td>( k_{\text{on}} ) min(^{-1} )</td>
<td>First-order dissociation constant</td>
<td>0.0153 (206)</td>
</tr>
<tr>
<td>( k_{\text{int}} )</td>
<td>Internalization rate constant</td>
<td>0.0966 (38)</td>
</tr>
<tr>
<td>( k_{\text{off}} ) min(^{-1} )</td>
<td>Absorption rate constant at 0.5,nmol</td>
<td>0.00820 (9)</td>
</tr>
<tr>
<td>( k_{\text{off}} ) min(^{-1} )</td>
<td>Absorption rate constant at 5,nmol</td>
<td>0.00579 (11)</td>
</tr>
<tr>
<td>( k_{\text{inG}} )</td>
<td>Absorption constant at 50,nmol</td>
<td>0.00273 (11)</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>1 (fixed)</td>
<td></td>
</tr>
<tr>
<td>Total receptor concentration</td>
<td>5.21 (5)</td>
<td></td>
</tr>
</tbody>
</table>
increasing doses. The NCA results from the mean profiles of exendin-4 are summarized in Table 1. Slope parameters $\lambda_1$, $\lambda_2$, and $CL$ decreased with increasing exendin-4 doses. In general, because of the limited target-binding capacity, drugs exhibiting TMDD show saturable distribution, with a decrease in apparent distribution parameters ($V_{ss}$ and $k_{on}$) with increasing doses. However, this trend was observed in $CL_D$ but not in $V_{ss}$ for exendin-4 in rats. GLP-1Rs are widely expressed in nonpancreatic tissues, and these receptors may also contribute as binding sites for exendin-4, thus making changes in $V_{ss}$ not evident. In addition, the apparent steady-state concentrations ($C_{ss}$) resulting from continuous infusion were not dose-proportional. After subcutaneous injection, terminal half-lives were 120 to 200 min, indicating involvement of flip-flop kinetics.

To detect and properly quantify nonlinearities in PK, a wide range of drug doses is required. In humans, disposition of exendin-4 has been described as linear over a narrow dose range, and the nonlinearity might also be hidden by flip-flop kinetics after subcutaneous injection. Straightforward evidence of nonlinear kinetics in rats was the lack of dose-proportionality of NCA parameters and $C_{ss}$ resulting from continuous infusion (Table 1).

Early blood sampling is particularly important to capture binding characteristics of drugs with the general TMDD model, and concentrations around the $K_D$ value are desired. In the rat study, data were available from 5 min after intravenous bolus dosing and via various administration routes, and observed concentrations ranged widely around the $K_D$ value.

All parameters were estimated (Table 2) with reasonable precision (<50% except for $k_{on}$). The clearance ($CL = k_{el} \times V_c$) is 3.62 ml/min, which is very close to the reported renal clearance (3.44 ml/min) (Parkes et al., 2001a). The equilibrium dissociation constant $K_D$ ($= k_{off}/k_{on}$) is 0.74 nM is in the range of the reported values for specific binding of exendin-4 and GLP-1 to normal rat tissues (Göke et al., 1993, 1995; Larsen et al., 1997; Satoh et al., 2000). The total receptor concentrations ($R_{tot}$) were estimated to be 5.21 nM for SD rats. The internalization rate constant was slightly higher than $k_{el}$. Bioavailability was estimated as close to 1 and then fixed as 1 in the final model. The absorption rate constant in rats decreased with dose, as observed in rats.

**Monkey PK.** The fitted profiles of exendin-4 in monkeys after various intravenous and subcutaneous doses are presented in Fig. 3, and parameter estimates are listed in Table 3. The model well described monkey PK profiles and yielded parameter estimates with reasonable CV%, except for parameters related to the drug-receptor complex ($k_{on}$, $k_{off}$, and $k_{int}$). The value of $CL_c$ was 2.39 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$, within the range of glomerular filtration rate (GFR) in healthy monkeys (2.2–3.6 ml $\cdot$ kg$^{-1} \cdot$ min$^{-1}$) (Altaian and Dittmer, 1974). The $K_D$ value in monkeys was 0.12 pM, which is 5000-fold lower than that estimated in rats. The internalization rate constant in monkeys was 15 times lower than $k_{el}$. Bioavailability was around 70%, and the absorption rate constant decreased with dose, as observed in rats.

**FIG. 4.** Exendin-4 concentration versus time profiles after single subcutaneous doses (top, study A; middle, study B) and iv infusion (bottom, study C) in humans. Symbols are mean drug concentrations with error bars representing S.E ($n = 7–8$), and solid lines are fitted profiles.
Human PK. The model-predicted time profiles of exendin-4 in three human studies are shown in Fig. 4, and parameter estimates are listed in Table 3. The TMDD model adequately described the PK profiles from the three studies but was unable to estimate total receptor content (R<sub>tot</sub>); thus, this parameter was fixed as a computer generalized value of 1.24 nM. This value is 3.2-fold lower than the R<sub>tot</sub> estimate in rats, which agrees with one observation where rat receptor density in lung and thyroid gland was 2- to 5.5-fold that of human receptor density (Körner et al., 2007). Because concentrations only after subcutaneous doses and one continuous intravenous infusion dose were available, we were not surprised that parameters were estimated with generally high CV%, especially for k<sub>on</sub>, k<sub>off</sub>, and k<sub>int</sub>. The CL<sub>c</sub> was 1.48 ml·min<sup>-1</sup>·kg<sup>-1</sup>, almost identical to the GFR in healthy subjects (125 ml/min in a 70-kg human). Bioavailability was fixed at 1, and the absorption rate constants were lower at higher doses, as was also found in other species. A population modeling approach with more extensive data would improve these parameter estimates.

Pharmacodynamics. Increases of glucose and insulin after glucose challenge during continuous infusion of exendin-4 in rats are shown in Fig. 5. Because no drug concentrations were measured in this study, the drug PK profiles (Fig. 6, top) were simulated according to the TMDD model and parameter values. Exendin-4 almost (>80%) reached steady state after 30 min of infusion, at which time glucose was injected.

The next step was to evaluate the appropriateness of the feedback model describing the glucose and insulin physiological system in rats. The model reasonably characterized the glucose and insulin profiles, and all parameters, except S<sub>Glu</sub>, had similar values between control and other dose groups. Therefore, the feedback model well represents the glucose-insulin system in rats. The initial analysis also showed that drug treatment affected only S<sub>Glu</sub>, the stimulation factor of glucose on insulin production, which was in agreement with the mechanism of action of exendin-4 on beta cells. Thus, in the next step of modeling, all rats shared the same set of parameters, but S<sub>Glu</sub> was allowed to change. As shown in Fig. 6, bottom, the estimated S<sub>Glu</sub> values first increased and then decreased with drug concentrations at the time of glucose challenge (C<sub>30min</sub>). The Adair function (Adair, 1923) was able to characterize the bell-shaped relationship between S<sub>Glu</sub> and C<sub>30min</sub>, and Fig. 6, top, depicts the fitted curve with this function.

The drug-RC was also tested as the driving force for PD. The model fitted the concentration profiles well but failed to generate precise parameters (ADAPT II was not able to provide CV% for parameter

![Fig. 5. Time profiles of glucose (left) and insulin (right) concentrations in rats during saline (A) or drug (3 (B), 30 (C), 300 (D), and 3000 (E) pmol·kg<sup>-1</sup>·min<sup>-1</sup>) infusions with glucose bolus challenge at 30 min. Symbols are mean concentrations with error bars representing S.E. (n = 4–8), and solid lines are fitted profiles.](image-url)
Pharmacokinetics. The binding of exendin-4 to GLP-1R can be saturated at high ligand doses, which contributes to the saturable clearance of exendin-4, although this has not been experimentally quantified. We reported that exendin-4 followed TMDD kinetics in Goto-Kakizaki (GK) diabetic rats (Gao and Jusko, 2011). In general, the parameter estimates were similar between SD rats (in the current report) and GK rats. Comparisons of the two rat strains were described previously (Gao and Jusko, 2011).

The primary elimination route of exendin-4 has been proposed as glomerular filtration, and $k_{\text{off}}$ physiologically represents renal elimination (Copley et al., 2006). The linear $CL_b$ in rats, monkeys, and humans was very close to reported renal clearances or GFR in healthy species. In rats, the relative contribution of $CL_b$ to the total clearance was approximately 73% at the lowest dose and nearly 100% at the highest doses. The $V_s$ in the three species was larger than plasma (serum in monkeys) volume. Simple allometry was assessed for various parameters. Figure 7 shows the correlation between selected PK parameters ($CL_b$, $V_s$, and $k_{\text{off}}$) and body weights. The allometric exponents for $CL_b$, and $k_{\text{off}}$ are close to 0.75, and that for $V_s$ is close to 1, similar to typical theoretical values. As was observed for type I interferon PK (Kagan et al., 2010), other PK parameters did not show a meaningful trend with body weight.

The GLP-1R undergoes endocytosis, and in the presence of agonist, the receptor cycles between the plasma membrane and endosomal compartment. The internalization of rat GLP-1R was examined in cell lines, with $k_{\text{on}}$ as 0.082 l/min$^{-1}$, nM$^{-1}$ and $k_{\text{off}}$ as 0.015 and 0.21 min$^{-1}$ (Widmann et al., 1995). Our estimated $k_{\text{on}}$ was approximately 4 times lower than these measured values, and $k_{\text{off}}$ was identical to the lower value, which resulted in a $K_D$ value (0.74 nM) comparable to that reported previously (Göke et al., 1995; Larsen et al., 1997; Satoh et al., 2000). The internalization half-life was 7 min (0.693/$k_{\text{on}}$) longer than the reported 2 to 3 min. The discrepancy could be due to the difference between in vivo and in vitro experiments. According to the authors, parts of the receptors continuously recycle back to the cell surface with a half-life of 15 min, without involvement of newly synthesized protein in a significant manner. This process was also handled in the model by using a fixed total receptor concentration ($N_{\text{total}}$) without a production and degradation process. However, the fraction of recycled GLP-1R was not considered in the current model. The average expression level of the receptors was 14.8 pmol/g (5.206 nM/350 g) tissue assuming receptors were evenly distributed throughout the tissues. Göke et al. (1995) reported the binding sites of exendin-4 on the posterior lobe of rat pituitary as 7.8 pmol/g tissue, similar to our estimates, especially considering that other tissues (e.g., islets, intestine) may have higher GLP-1R density (Körner et al., 2007).

Different values of $K_D$ for exendin-4 in animals and humans have been reported depending on whether a single class or two binding sites were assumed and the cell lines tested. Our estimated human $K_D$ (1.38 nM) was higher than the reported value of 0.82 nM for GLP-1 binding to human pituitary membranes (Satoh et al., 2000). The rat and human GLP-1R exhibit 95% amino acid homology and are 90% identical (Doyle and Egan, 2007), and the $K_D$ value in rats is 53% of the values in humans. The $K_D$ in monkeys was 0.01% of the other two species and was close to the lower $K_D$ of exendin-4 binding to the rat posterior lobe (Göke et al., 1995). Nevertheless, $k_{\text{on}}$ and $k_{\text{off}}$ in monkeys and humans are not precisely estimated.

Pharmacodynamics. The PD component of the model reflects the stimulation of insulin secretion by exendin-4. Exendin-4 has to dis-
tribute to pancreas and bind to GLP-1R to stimulate insulin release. However, pancreatic GLP-1R is mostly expressed on the surface of the beta cells facing the endothelium (Tornehave et al., 2008), and distribution would be quite fast; therefore, a biophase between plasma and pancreas is unnecessary.

One can argue that exendin-4 stimulates insulin secretion by binding to pancreatic GLP-1R and then initiating receptor-mediated signaling pathways. However, in the final PK/PD model, the plasma concentration ($C$), rather than drug-RC, was found to work better as the driving force for exendin-4 insulinotropic effects. Mathematically, if the effect is driven by $C$ (eq. 7), the transduction between receptor binding and effect is assumed as linear. If the effect is initiated by RC (eq. 8), the transduction between receptor binding and the effect is implied as nonlinear.

Direct evidence supporting eq. 7 over eq. 8 came from our observations in GK rats (Gao and Jusko, 2011). After an intravenous bolus of exendin-4, insulin peaked before the maximal concentrations of RC. Moreover, because pancreatic GLP-1Rs are mostly expressed on the surface of the beta cells (Tornehave et al., 2008), exendin-4 can bind to the receptor quite fast and directly stimulate insulin release. Furthermore, binding in other tissues besides pancreas might account for a large portion of drug-receptor complexes.

The basic structure of the PD model represents the feedback mechanism between glucose and insulin. This model uses the most simplistic mechanism and functions adequately in various situations. In general, the final estimated parameters controlling glucose and insulin regulation (Table 4) are in accordance with literature values (Jin and Jusko, 2009).

The likely mechanism of action of exendin-4 was integrated into the feedback model using $S_d$ as shown in eq. 7. The stimulation of exendin-4 on insulin secretion is glucose-dependent (Doyle and Egan, 2007). Only when glucose concentrations are higher than a certain threshold is the insulinotropic effect evident. This phenomenon was also observed in the rat study: glucose and insulin concentrations did not change during the first 30 min of exendin-4 infusion. This dependence was modeled as the difference of glucose over basal concen-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Estimate</th>
</tr>
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<tbody>
<tr>
<td>$k_{outG}$</td>
<td>1/min</td>
<td>0.046 (9)</td>
</tr>
<tr>
<td>$k_{outI}$</td>
<td>1/min</td>
<td>0.483 (50)</td>
</tr>
<tr>
<td>$S_{ins}$</td>
<td>nM$^{-1}$</td>
<td>0.157 (46)</td>
</tr>
<tr>
<td>$S_{Glu}$</td>
<td>mM$^{-1}$</td>
<td>0.0684 (20)</td>
</tr>
<tr>
<td>$V_g$</td>
<td>l/kg</td>
<td>0.208 (5)</td>
</tr>
<tr>
<td>$S_{max}$</td>
<td>Maximal response factor</td>
<td>4.67 (30)</td>
</tr>
<tr>
<td>$k_i$, nM</td>
<td>First receptor binding constant</td>
<td>0.826 (71)</td>
</tr>
<tr>
<td>$k_2$, nM$^{-1}$</td>
<td>Second receptor binding constant</td>
<td>0.0153 (69)</td>
</tr>
</tbody>
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trations. When glucose is not higher than basal values, the effect is shut off. The dose-response relationship of exendin-4 both in vitro (Parkes et al., 2001b) and in vivo was reported as a bell-shaped curve. Consistent with the initial PD analysis, $S_{\text{Glu}}^{\text{peak}}$ increased with dose until 300 pmol·kg$^{-1}$·min$^{-1}$ and then dropped to a lower value at 3000 pmol·kg$^{-1}$·min$^{-1}$. The exact reason for this bell-shaped dose-response relationship is not clear, but the Adair function adequately captured the relationship between $S_{\text{Glu}}^{\text{peak}}$ and plasma concentrations at 30 min (Fig. 6, top). Subsequently, the Adair function was directly incorporated into the feedback model to form the PK/PD model. According to the simulations (Fig. 8), maximal insulin secretion with this experimental design would be reached at an infusion rate of 120 pmol·kg$^{-1}$·min$^{-1}$.

Because the Adair function was originally proposed to characterize multiple binding sites on a single receptor, there might be a possibility that a second exendin-4 molecule can bind to the exendin-4-GLP-1R complex, probably when the complex is recycled back to the cell surface. It may be feasible to express the pancreatic GLP-1R as some portion of the total receptor pool and the insulinotropic effect proportional to 1:1 drug-receptor complex. To be more mechanistic, the 1:1 complex can be internalized or dissociated to free receptor with the first dissociation rate constant ($k_1$) or interact with another exendin-4 molecule with the second dissociation rate constant ($k_2$).

Other mathematical functions might also describe the bell-shaped dose-response relationship, such as a hypothetical antagonist effect generated by exendin-4. However, physiologically, this is not detected in rats, and mathematically, more parameters in the model would lead to over-parameterization. Therefore, the simple Adair function is reasonable for modeling the insulinotropic effects of exendin-4.

In conclusion, a mechanistic TMDD PK/PD model was developed that provides quantitative insights into the in vivo PK properties of exendin-4 in various species and the in vivo PD properties in healthy rats. Plasma clearance and volume of distribution followed simple allometric scaling principles across species. The integrated PK/PD model was exemplified using data from healthy rats and well described glucose and insulin response profiles. This model may prove useful in future animal and clinical studies of exendin-4 and other GLP-1 derivatives.

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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.

References

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