

# A Quantitative Look into the Effect of Glucose on Glucokinase Function in the Presence of an Activator

Michael G. Zager\*, Shaoxian Sun and Kathleen M. Ogilvie  
Pfizer Global Research and Development, San Diego, CA

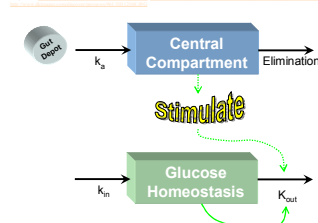
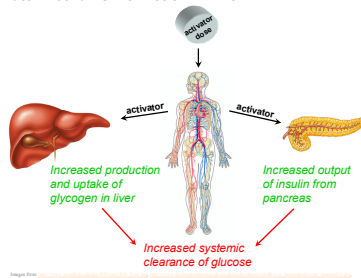


## Glucokinase Background / Project Objective

- Glucokinase (GK) is an allosteric enzyme that regulates systemic glucose levels via glucose phosphorylation
  - Liver: Glycogen production and uptake
  - Pancreas: Insulin output
- Biochemical activity of GK is significantly dependent on concentration of glucose present (positive feedback)
- Biochemical  $K_m$  of GK for glucose is hypothesized to be the controller of natural *in vivo* glucose set point
- GK activators bind to allosteric site causing a sustained activated state  $\rightarrow$  higher level of total activation relative to glucose present
- Biochemically, this left-shifts the  $K_m$  value
  - Effectively increases glucose clearance rate
- Possible treatment for diabetes
- Objective of this effort:** Investigate how the dependence of GK activity on glucose affects the overall glucose-lowering efficacy of a GK activator in a diabetic animal model

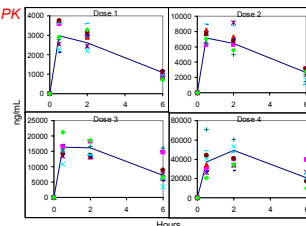
## Modeling Methods

### GK Activator Mechanism of Action *in vivo*

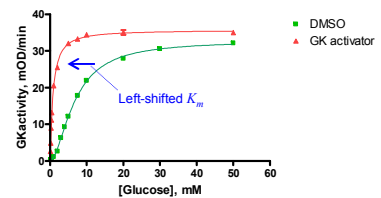


### Model Calibration to *in vivo* study

- Acute dose-response study
  - 4 dose levels, oral
  - N = 9
  - Plasma PK and glucose analyzed
- Model fitting
  - NONMEM
  - Naïve pool analysis
  - Sensitivity analysis  $\rightarrow E_{max}$  and  $EC_{50}$  estimates robust



### Biochemical Behavior of GK with an Activator

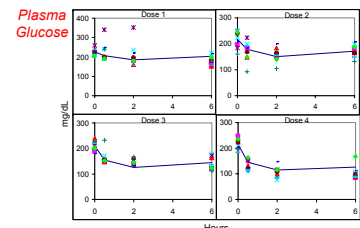


- Baseline (DMSO) parameters for model:  $V_{max}$ ,  $K_m$ , Hill coefficient
- Expressions for biochemical relationship between GK activity, glucose and activator:

$$\frac{V_{max} \cdot C_{PS}}{(K_m \cdot f(C_p))^n + C_{PS}} \quad f(C_p) = 1 - \frac{E_{max} \cdot C_p}{EC_{50} + C_p}$$

- Model assumption:** Dynamics of systemic glucose regulation are mainly controlled by the biochemical relationship between GK and glucose

$$\frac{dC_{PS}}{dt} = k_{in} - k_{out} \cdot \frac{C_{PS}}{(K_m \cdot f(C_p))^n + C_{PS}}$$



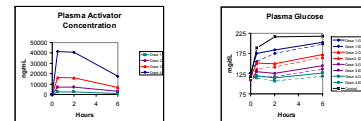
## Model Analysis / Simulations

### Dynamical Behavior of Model $\rightarrow$ Limitations in Predicting

$$\frac{dC_{PS}}{dt} = k_{in} - k_{out} \cdot \frac{C_{PS}}{(K_m \cdot f(C_p))^n + C_{PS}}$$

- Parameter  $k_{out}$  is unknown  $\rightarrow$  estimated using *in vivo* data
- Parameter  $k_{in}$  is a function of  $K_{out}$  and the initial value of plasma glucose
  - Ensures glucose homeostasis ( $G_{eq}$ ), assuming  $G_{eq}$  = initial value
- Simulations with these values for  $k_{in}$ ,  $k_{out}$  and  $G_{eq}$  fixed but varying the initial value for glucose is within model limitations
- Simulations with varying  $G_{eq}$  values is out of model limitations
  - This will change the speed of glucose dynamics governed by the magnitude of parameters  $k_{in}$  and  $k_{out}$

### Simulations – Glucose Dependence vs. Standard IDR Model



- Standard Indirect Response (IDR) model was calibrated to data
- Initial glucose values set to ~50% baseline (nearly maximum observed effect)
- Head-to-head simulations were run using the glucose dependent model (GDM) and the IDR model
- IDR model predicts lower sustained glucose values after activator dose
- Largest differences occur at smaller (clinically relevant) doses, where maximum difference approximately coincides with maximum activator concentration

## Conclusions

- Assuming GDM is a more accurate model than IDR, simulations suggest activators targeting GK may lose potency with relatively low plasma glucose
- As a target for diabetes treatment, this suggests the question, "Is it possible to manipulate this target with a drug such that efficacy is obtained while protecting against risk of treatment-induced hypoglycemia in patients?"
- Future work includes investigation of this question by building more mechanistic granularity into the model
- Greater mechanistic reality reduces model limitations  $\rightarrow$  increases space of confidence for testing and generating hypotheses through simulation and experimentation

## Acknowledgements

- in vitro* – Tom Carlson
- in vivo* – Bernadette Pascual, Sharon Lostracco, Kirk Kozminski, Natalie Hosea
- Chemistry – Jihong Lou, Anthony Ling, Paul Humphries

## Wet Lab Experimentation / Motivation for Model

### *in vitro*

- We investigated GK biochemical activity in presence of varying concentrations of glucose and glucose plus a GK activator
- Results confirmed a sigmoidal kinetic (cooperative) relationship between glucose concentration and GK biochemical activity
- Activator presence resulted in a significantly left-shifted  $K_m$  value of the curve; minimal  $V_{max}$  modulation

### *in vivo*

- We tested the activator for its ability to reduce plasma glucose levels in an acute dose-response study using Diet-Induced Obese (DIO) mice
- Robust exposure-response relationship observed
- Glucose modulations range from minimal to clear maximum response