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ORIGINAL ARTICLE

Pharmacokinetic and pharmacodynamic modelling of the effects of glimepiride on insulin secretion and glucose lowering in healthy humans

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SUMMARY

Glimepiride is an oral sulfonylurea antihyperglycaemic agent. We used pharmacokinetic–pharmacodynamic (PK–PD) modelling to analyse the relationship between plasma glimepiride concentration, insulin secretion and glucose lowering to determine the effects of the drug in healthy volunteers. A single 2-mg oral dose of glimepiride was administered to six healthy volunteers. The control group received a placebo. All subjects consumed 12 g of sugar immediately after drug administration in order to standardize the initial plasma glucose levels. Serial blood sampling was performed for 9 h after oral dosing. Plasma glimepiride, insulin and glucose levels were determined by validated methods (LC/MS/MS assay, hexokinase method and radioimmunoassay respectively). Time courses of plasma glimepiride concentration, insulin secretion, and glucose lowering effects were analysed by means of PK–PD modelling with the ADAPT II program. The time course of the plasma concentrations followed a two-compartmental model with a lag time. The glimepiride concentration peaked at 191·5 ng/mL at approximately 4 h after administration. The maximal increase in insulin secretion was 9·98 mIU/L and the maximal decrease in plasma glucose was 19·33 mg/dL. Both peak effects occurred at approximately 2·5 h after drug intake. The glucose disappearance model was used to analyse glimepiride's insulin secretion and glucose lowering effects. The PK–PD model described well the relationship between plasma glimepiride and its insulin secretion and hypoglycaemic effects in healthy volunteers.

Keywords: **glimepiride, pharmacodynamic, pharmacokinetic, pharmacokinetic–pharmacodynamic modelling**

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INTRODUCTION

Glimepiride, 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl] phenyl]sulfonyl]-3-(*trans*-4-methylcyclohexyl) urea, is an oral sulfonylurea antihyperglycaemic agent that contains a sulfonylurea nucleus and a cyclohexyl ring (1). Glimepiride may be given once daily. It has a long-lasting effect without markedly increasing plasma insulin compared with other sulfonylureas (2). Glimepiride's major site of action is thought to be a membrane receptor on pancreatic β-cells, where it acts via ATP-regulated potassium (K_{ATP}) channels to cause membrane depolarization and insulin release (3). The association rate of glimepiride is 2·5- to 3-fold that of glibenclamide, its dissociation rate is 8- to 9-fold that of glibenclamide, and its *in vitro* binding affinity for rat β-cell tumour and insulinoma cells is 2·5- to 3-fold lower than that of glibenclamide (4). Sulfonylureas interact with different sites on the pancreatic β-cell membrane. Glimepiride binds to a 65-kDa protein, whereas glibenclamide binds to a 140-kDa protein. These proteins are believed to both be part of the same sulfonylurea receptor, as each agent inhibits the other's binding to its protein target (5).

In single-dose studies with healthy volunteers, the peak glimepiride plasma concentration (C_{max}) and the area under the plasma concentration–time curve (AUC) were generally dose-proportional. In 12 healthy volunteers, the C_{max} rose linearly from

103·2 to 550·8 μg/L as the dose increased from 1 to 8 mg, whereas the AUC increased from 339 to 2634 μg h/L. The glimepiride C_{max} occurred at 0·7–2·8 h (T_{max}) after the single-dose administration to healthy volunteers (6). Glimepiride plasma protein binding was 99·4% (7), and the volume of distribution (V_d) was 8·8 L (8).

From dose-ranging studies in patients with type 2 diabetes mellitus, glimepiride appears to reduce fasting and postprandial blood glucose levels, as well as glycosylated haemoglobin. These effects are dose-dependent over a range of 1–4 mg daily. For patients receiving the maximum daily dose (8 mg), the average reduction in glycosylated haemoglobin is 2% in absolute units. Age, gender, weight, and race do not affect glimepiride's efficacy (9).

The main objective of this study was to examine the relationship between plasma glimepiride concentration and its insulin secretion and glucose lowering effects (i.e. the increase in blood insulin and decrease in blood glucose) after its oral administration to healthy volunteers. This should permit prediction of the time course of glimepiride's therapeutic and side effect profiles after oral dosing. The relationship between the pharmacokinetics of glimepiride and its insulin secretion and glucose lowering effects has not yet been analysed. Our goal was to assess the usefulness of pharmacokinetic–pharmacodynamic (PK–PD) modelling in describing this relationship.

METHODS

Subjects

Six healthy male subjects with a mean age of 25 years (range = 22–25 years) and a mean weight of 69·67 kg (range = 55–83 kg) took part in this study. All subjects underwent a thorough history, a complete physical examination, and a battery of routine laboratory tests (haematology, serum chemistry and urinalysis). None had taken any drugs known to interfere with the study for at least 10 days beforehand. The exclusion criteria included health problems, drug or alcohol abuse, and abnormalities in laboratory screening tests. All subjects were told the full details of the study and gave written informed consent. The study was approved by the local ethics committee.

Study design

All subjects fasted for at least 10 h before taking their study medication. At time zero, an intravenous cannula was inserted into the forearm vein and control blood samples were collected.

First period, six subjects received a single-oral dose Amaryl 2 mg and second period, same subjects received a placebo. There was a 6-day washout period between the periods. After baseline sampling, the test group took glimepiride (Amaryl® 2 mg tablet; Handok/Aventis Pharma Co. Ltd, Seoul, Korea) with 240 mL of water. The control group took a placebo with 240 mL of water. All subjects consumed 12 g of sugar cubes immediately after drug administration in order to prevent hypoglycaemia and maintain standard initial plasma glucose level. All subjects were given a standardized meal 4 h after drug administration. They were not allowed to remain supine or to sleep until 4 h after drug administration.

Samples for plasma glimepiride, insulin and glucose determinations were taken at 0·5, 1, 1·5, 2, 2·5, 3, 4, 5, 7 and 9 h after drug administration. The samples were collected in heparinized tubes, immediately centrifuged (10 min at 1650 *g*), and stored at −80 °C for later analysis.

Plasma assay

Plasma glimepiride was assayed by the reported LC/MS/MS method, with a slight modification (1). Briefly, 50 μL of internal standard (glibenclamide, 500 ng/mL) and 0·5 mL of 1 M NaOH were added to 0·5 mL of plasma, followed by a 10-min liquid–liquid extraction with 5 mL of ethyl ether : ethyl acetate (1 : 1, v/v). The organic layer was separated and evaporated to dryness at ambient temperature in a Speed-Vac (Savant, Holbrook, NY, USA). The residue was reconstituted in 100 μL of acetonitrile by vortexing for 15 s; then 5 μL of this solution was injected onto the column. The mobile phase was a mixture of 0·1% formic acid buffer : acetonitrile (20 : 80, v/v), and the column was eluted at 0·2 mL/min with an HP 1100 series pump (Agilent, Wilmington, DE, USA). The turbo-ion spray interface was operated in positive ion mode at 5500 V and 350 °C. Using flow injection of a mixture of all analyses, the operating conditions were optimized to: nebulizing gas flow, 1·04 L/min; auxiliary gas

flow, 4·0 L/min; curtain gas flow, 1·44 L/min; orifice voltage, 80 V; ring voltage, 400 V and collision gas (nitrogen) pressure, $3{\cdot}58 \times 10^{-5}$ Torr. Quantitation was performed by multiple reaction monitoring of the protonated precursor ion and the related product ion for glimepiride, using the internal standard method with the peak area ratio. The mass transitions used for glimepiride and the internal standard were m/z 491·0 → 352·4 and 494·0 → 369·4 respectively (20 eV collision energy, 200 ms dwell time). Quadruples Q1 and Q3 were set on unit resolution. The analytical data were processed using Analyst Software (version 1·2; POET software corporation, London, UK).

For all plasma samples, glucose concentrations were determined enzymatically by the hexokinase method, and insulin concentrations were determined by radioimmunoassay (10).

Pharmacokinetic/pharmacodynamic model and data analysis

Pharmacokinetics (plasma glimepiride) and pharmacodynamics (plasma insulin and glucose) were modelled sequentially. We developed a parsimonious compartmental model that reflects the rate of change of glucose as the difference between the net hepatic glucose balances. As shown in Fig. 1, a two-compartment model with a lag time, nonlinear absorption and elimination was selected as the most appropriate pharmacokinetic model to reflect the oral administration and flip–flop kinetics. Model development was an iterative process with regard to both the underlying data set and the selected model structure. Models were constructed as a series of differential equations that were solved numerically and fitted to the data with the ADAPT II software (Biomedical Simulation Resource, Los Angeles, CA, USA) (11). The fitting with individual data was performed by means of maximum likelihood estimation. The following information was used to evaluate the goodness of fit and the quality of the parameter estimates: coefficient of variation of parameter estimates, parameter correlation matrix, sums of squares of residuals, visual examination of the distribution of residuals and the Akaike Information Criterion (12). The differential equations that described the changes in the amounts of glimepiride in the compartments after oral administration are given by Eqs (1) to (3):

$$\frac{dx_1}{dt} = -\left(\frac{V_{max12}}{K_{m12} + x_1}\right) \times x_1 \quad (1)$$

$$\frac{dx_2}{dt} = -\left(\frac{V_{max12}}{K_{m12} + x_1}\right) \times x_1 + k_{32} \times x_3 - K_{23} \times x_2 - \left(\frac{V_{max2e}}{K_{m2e} + x_2}\right) \times x_2 \quad (2)$$

$$\frac{dx_3}{dt} = K_{23} \times x_2 - K_{32} \times x_3 \quad (3)$$

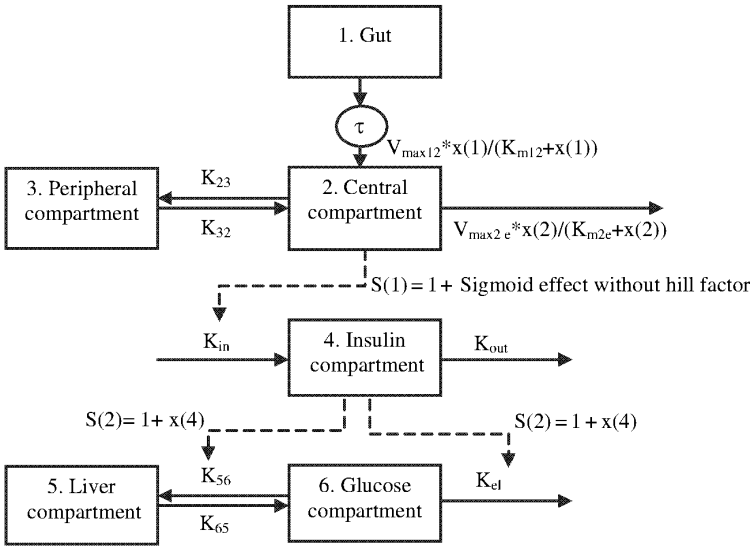

Fig. 1. The model selected to describe the effects of glimepiride on insulin secretion and glucose lowering in healthy volunteers: τ, absorption lag time; V_{max} and K_{mij}, Michaelis–Menten transport parameters; K_{ij}, first-order rate constant; K_{in}, zero-order rate constant for insulin production; K_{out}, the first-order constant for insulin loss.

First-order rate constants describing intercompartmental transport are denoted by K_{ij}, and the active transport with Michaelis–Menten type kinetics is characterized by the apparent maximal transport rates V_{maxij} and the apparent Michaelis constants K_{mij} (13). After the parameters of the pharmacokinetic model were fixed, the model served as an input function for the pharmacodynamic models (14).

An insulin-dependent glucose disappearance model was used to analyse the PK–PD relationship. (15) In our study, the response variables measured were the insulin plasma concentration (milli-international units per litre, mIU/L) and the glucose plasma concentration (mg/mL). The system of differential equations shown below describes the model:

$$\frac{dx_4}{dt} = K_{in} \times S_1(t) - K_{out} \times R \quad (4)$$

$$\frac{dx_5}{dt} = K_{65} \times x_6 - K_{56} \times S_2(t) \times x_5 - K_{6e} S_2(t) \times x_5 \quad (5)$$

$$\frac{dx_5}{dt} = K_{56} \times S_2(t) \times x_5 - K_{65} x_6 \quad (6)$$

$$s_1(t) = 1 + \frac{s_{max} \times (x_2/V_2)}{SC50 + (x_2/V_2)} \quad (7)$$

$$S_2(t) = 1 + x_4 \quad (8)$$

$$R_0 = \frac{K_{in}}{K_{out}} \quad (9)$$

where K_{in} represents the zero-order constant for production of the insulin response and K_{out} defines the first-order rate constant for loss of the insulin response. In our study, a model that assumed glimepiride simulated K_{in} was considered to be closest to the pharmacological action of the drug. However, both modelling approaches, with glimepiride inhibiting K_{out} or stimulating K_{in}, were investigated to compare the performance of the model and the physiologic relevance of the parameters obtained.

In this model, the rate of change of plasma glucose is the difference between the net hepatic glucose balance and the disappearance of glucose into peripheral tissues only (16). We have shown that the hepatic glucose balance varies according to the relationship shown in Eq. (5). To explain glucose disappearance, it is assumed that insulin acts from a remote compartment, that insulin increases the mobility of glucose across cell membranes, and that glucose mobility potentiates glucose disappearance. When insulin increases, it stimulates K_{56} and K_{60} according to the stimulation function given in Eq. (8).

RESULTS

Pharmacokinetic analysis

The mean plasma concentration-vs.-time curve after oral administration of 2 mg of glimepiride is shown in Fig. 2, where the solid line represents the best fit of the pharmacokinetic model to the measured concentration, based on the means of the individual parameter estimates. Based on the maximum likelihood criterion and visual inspection of the fits, a two-compartment model with a lag time, nonlinear absorption and elimination was chosen to describe the data. The estimated pharmacokinetic parameters are listed in Table 1: The Michaelis–Menten type absorption rate into the central compartment V_{max12} and the apparent Michaelis constant K_{m12} equal 1042·58 ng/h and 70·31 ng respectively. The Michaelis–Menten kinetic analysis of the data indicated the existence of a second carrier-mediated transport process and of an interaction between sulfonylurea and highly protein-bound drugs that govern elimination from the central compartment (V_{max2e} = 0·14 ng/h, K_{m2e} = 0·006 ng) (2). The terminal elimination half-

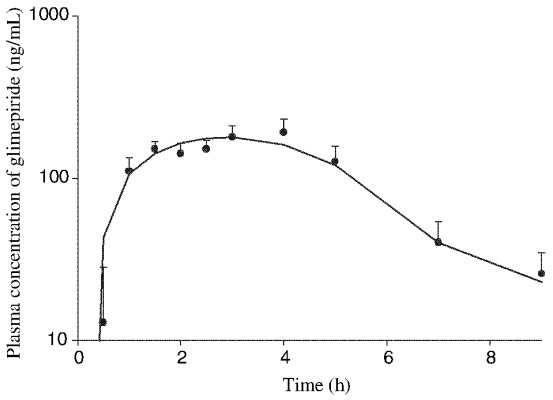

Fig. 2. Plasma glimepiride concentration after a single 2-mg oral dose to healthy volunteers (mean ± SEM, n = 6). Data points are observed values; the solid line is the result of maximum likelihood fitting with the ADAPT II program.

Table 1. Pharmacokinetic parameters for glimepiride after a single 2-mg oral dose in healthy volunteers (mean ± SEM, $n = 6$)

Parameter	Value
Model independent parameter	
AUC (ng h/mL)	1021·43 ± 199·55
C_{max} (ng/mL)	191·52 ± 29·80
T_{max} (h)	2·58 ± 0·52
CL(inf)/F (L/h)	2·26 ± 0·33
Vz(terminal)/F (L)	7·82 ± 1·22
$t_{1/2}$	2·55 ± 0·37
Model dependent parameter	
K_{m12} (ng)	70·31 ± 30·51
V_{max12} (ng/h)	1042·58 ± 161·82
K_{m2e} (ng)	0·006 ± 0·0004
V_{max2e}(ng/h)	0·14 ± 0·02
K_{23} (/h)	0·21 ± 0·11
K_{32} (/h)	0·72 ± 0·36
T_{lag} (h)	0·34 ± 0·04

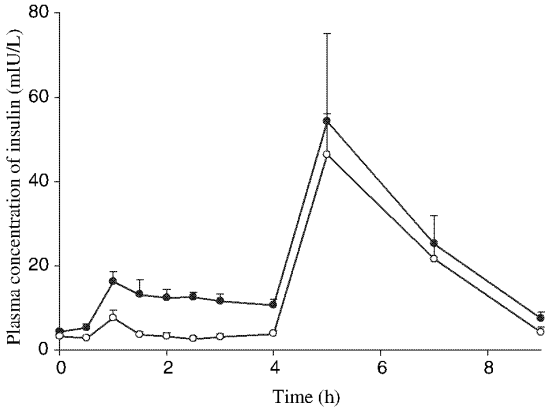

Fig. 3. Plasma insulin concentration after a single oral dose of placebo or 2 mg glimepiride to healthy volunteers (mean ± SEM, $n = 6$). Open circles, plasma insulin concentration after placebo. Closed circles, plasma insulin concentration after 2 mg glimepiride.

life ($t_{1/2\beta}$) was 2·548 ± 0·901 h and the CL_{total}/F was 2·262 ± 0·814 L/h. Our pharmacokinetic profile for glimepiride is similar to that found in other studies involving healthy volunteers and the same glimepiride dose (10).

Insulin secretion effect

Plasma insulin profiles after drug and placebo administration are shown in Fig. 3. In the control group, the maximal insulin secretion was 46·43 mIU/L at 5 h, and secretion returned to baseline at 9 h. In the test group, glimepiride caused a further significant increase (maximum increase of 9·98 mIU/L) in plasma insulin at between 1 and 5 h after its administration, and the insulin concentration returned to baseline by 9 h. Glimepiride produced a statistically significant increase in insulin secretion, relative to placebo, for 3 h (from 1 to 4 h after administration) ($P < 0·01$).

Glucose lowering effect

Figure 4 shows the glucose profiles after drug and placebo administration. The decline in plasma glucose was induced after a lag of approximately 1·5 h, and the decrease reached its maximum (21·00 mg/dL decrease in plasma glucose) approximately 2 h after dosing. Compared with the placebo group, glimepiride produced a statistically significant decrease in glucose levels for a period of 3·5 h, from 1·5 to 5 h after administration ($P < 0·01$).

Pharmacokinetic–pharmacodynamic modelling of insulin secretion and glucose lowering effects

The corresponding pharmacodynamic parameter estimates for plasma insulin and glucose are shown in Table 2. Figures 5 and 6 show the plasma insulin profile (post-drug level minus placebo level;

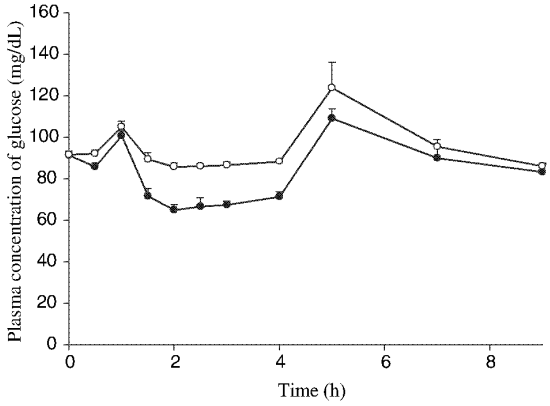

Fig. 4. Plasma glucose concentration after a single oral dose of placebo or 2 mg glimepiride to healthy volunteers (mean ± SEM, $n = 6$). Open circles, plasma glucose concentration after placebo. Closed circles, plasma glucose concentration after 2 mg glimepiride.

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