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Pharmacokinetic-Pharmacodynamic Modeling for the Relationship between Glucose-Lowering Effect and Plasma Concentration of Metformin in Volunteers

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Metformin is a biguanide antihyperglycemic agent often used for the treatment of non-insulin dependent diabetics (NIDDM). In this study, the pharmacokinetics and pharmacodynamics of metformin were investigated in Korean healthy volunteers during a fasting state for over 10 h. In order to evaluate the amount of glucose-lowering effect of metformin, the plasma concentrations of glucose were measured for a period of 10 h followed by the administration of metformin (oral 500 mg) or placebo. In addition, the concentration of metformin in blood samples was determined by HPLC assay for the drug. All volunteers were consumed with 12 g of white sugar 10 minutes after drug intake to maintain initial plasma glucose concentration. The time courses of the plasma concentration of metformin and the glucose-lowering effect were analyzed by nonlinear regression analysis. The estimated C_{max}, T_{max}, CL_t/F (apparent clearance), V/F(apparent volume of distribution), and half-life of metformin were 1.42 ± 0.07 μg/mL, 2.59 ± 0.18 h, 66.12 ± 4.6 L/h, 26.63 L, and 1.54 h respectively. Since a significant counterclock-wise hysteresis was found for the metformin concentration in the plasma-effect relationship, indirect response model was used to evaluate pharmacodynamic parameters for metformin. The mean concentration at half-maximum inhibition IC_{50}, k_{in}, and k_{out} were 2.26 μg/mL, 83.26 h^{-1}, and 0.68 h^{-1}, respectively. Therefore, the pharmacokinetic-pharmacodynamic model may be useful in the description for the relationship between plasma concentration of metformin and its glucose-lowering effect.

Key words: Metformin, Pharmacokinetics, Pharmacodynamics

INTRODUCTION

The biguanide metformin is an oral antihyperglycemic agent widely used in the management of non-insulin-dependent (type 2) diabetes mellitus (NIDDM) (McEvoy *et al.*, 2002). Metformin is used as monotherapy as an adjunct to diet for the management of type 2 diabetes mellitus in patients whose hyperglycemia cannot be controlled by diet alone. Metformin may also be used in combination with a sulfonylurea antidiabetic agent in patients with type 2 diabetes who do not achieve adequate glycemic control with the sulfoylurea agent alone (Kwon *et al.*, 2003). Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects (Paul *et al.*, 2001).

The main objective of this study was to examine the relationship between metformin plasma concentration and its glucose-lowering after oral administration to healthy volunteers. This should enable a prediction of the time-course of the therapeutic and side effect profiles of metformin for oral dosing strategies. Unfortunately, however, the relationship between the pharmacokinetic and the glucose-lowering effect of metformin has not been analyzed in the literature. Therefore, the objective of this study was to assess the applicability of pharmacokinetic-pharmacodynamic (PK-PD) modeling in he description of this relationship.

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MATERIALS AND METHODS

Subjects

Twenty two healthy male subjects with a mean age of 25.4 years (range 21-32 years) and a mean weight of 68 kg (range 55-89 kg) took part in this study. All subjects were selected after completing a through history and physical examination, and after a normal laboratory examinations which were consisted of hematology, 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 problem, drug or alcohol abuse and abnormalities in laboratory screening were exclusion criteria. All subjects were presented with full details of the investigation prior to consent. Each subject gave written informed consent to study procedures that were approved by the institutional review board of the Institute of Drug Development, Chungnam National University (Daejeon, Korea).

Study design

In this study, control group was used to calculate the difference of the plasma glucose level with or without the metformin administration. Eleven subjects of control group were selected from the test group.

All subject were fasted for at least 10 h prior to the timing of the dose. At time zero, an intravenous cannula was inserted into a forearm vein and blank blood samples were collected. After baseline blood sampling, metformin tablet (Glucophage, 500 mg) was orally given to the test group with 240 mL water. The control group received only 240 mL of water without the drug administration. All volunteers were consumed with 12 g of sugar after drug or water administration to maintain standard initial plasma glucose level. Blood samples for the determination of plama metformin were taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after drug administration. In addition, plasma glucose concentration was measured at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 h after the drug administration. All subjects abstained from food until the 4 h after the administration. The remaining blood samples were collected in heparinized tubes, immediately centrifuged for 10 min at 3000 rpm, and then stored at -20°C until HPLC analysis.

Determination of metformin and glucose in the plasma

Plasma metformin was measured by a validated HPLC method. Briefly, 800 μL of serum and 200 μL of internal standard (phenformin, 2 μg/mL in water) were mixed with 800 μL of deproteinizing solution (mixture of 0.5% zinc sulfate and 0.1% ethyleneglycol solution). The mixture was shaken vigorously for 30 minutes and centrifuged for 15 minutes at 3000 g. Then the upper aliquot was transferred to a vial and was injected 20 μL to the HPLC column. The separation was performed on a cation-exchange column (Nucleosil SA 100A, 4.6×250 mm I.D., 5 μm particle size) with an isocratic mobile phase consisting of 0.1 M tetramethylammonium phosphate buffer (pH 3.7)/ACN (80 : 20 v/v %). Quantification was achieved by UV detection at 236 nm. The detection limit of the assay was 0.1 μg/mL.

The plasma glucose concentration was determined by a glucose-oxidase/UV method (Stanbio Laboratory, Texas, USA). The calibration curve was linear (correlation coefficient, r=0.995) over the range of 0-500 mg/dL. The intra-day coefficients of variation were 1.6% and the inter-day coefficients of variation were less than 3.0% for plasma assays. The glucose-lowering effect of metformin [effect %] was calculated as the percentage change, at each collection time, from control group (PGc) glucose concentration of test group (PGt). This was calculated as follows, using obtained glucose concentration of control group :

$$\text{Effect}(\%) = \frac{PGc - PGt}{PGc} \times 100$$

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using non-compartmental and compartmental methods. The non-compartmental analysis was performed, using standard methods, for each subject. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule and extrapolated to infinity. We used a two-compartment model with first-order absorption and elimination that reflects the disposition kinetics of metformin. The model development was an iterative process, both with regard to the underlying data set and the selected model structures. Models were constructed as a series of differential equations that were solved numerically and fitted to the data using the ADAPT II-software package (D'Argenio & Schumitzky, 1997).

Fitting with individual data was performed using weighted least square estimation and assuming that the standard deviation of the measurement error is a linear function of the measured quantity. The following information (provided by ADAPT) was used to evaluate the goodness of fit and the quality of the parameter estimates: coefficients of variation of parameter estimates (CV), parameter correlation matrix, sums of squares of residuals, visual examination of the distribution of residuals, and Akaike information criterion (AIC). Note that drug input is assumed to occur in compartment 1, whereas compartments 2 and 3 represent the central compartment (distribution volume V2) and tissue regions for metformin disposition, respectively.

Pharmacodynamic analysis

A physiologic indirect response model with inhibition of the production of the response was thought to be appropriate to describe metformin pharmacodynamics. In the absence of a placebo effect, the differential equation for the effect (R) is :

$$\frac{dR}{dt} = Kin \cdot I(t) - Kout \cdot R$$

with

$$I(t) = 1 - \frac{Cp}{IC_{50} + Cp}$$

in which k_{in} is the apparent zero-order rate constant or the production of the drug response, and k_{out} is the first order rate constant for the disappearance of the response, I(t) is the inhibition function, and IC_{50} is the metformin concentration that produces 50% of maximum inhibition achieved at the effect site.

To evaluate possible hysteresis between the pharmacodynamic effect and metformin plasma concentrations, the effect was plotted against the concentration, and the data points were connected in time sequence. These plots display a counterclockwise hysteresis (Fig. 3). This temporal dissociation between the time courses of concentration and effect might be applied by mechanism-based indirect response model.

RESULTS

Pharmacokinetic analysis

The mean plasma concentration versus time curve after oral administration of 500 mg metformin is shown in Fig. 1. The solid line in Fig. 1 represents the best fit of the PK model to the measured concentrations, calculated from the parameter estimates and equations shown in the previous section. A two-compartment open model was chosen to describe the data based on the weighted least-squares (WLS) criterion and visual inspection of the fits. The estimated PK parameters are listed in Table 1. In this study, the nonlinear regression analysis yielded C_{max}, T_{max}, and CL_t/F estimates for 1.42 ± 0.07 μg/mL, 2.59 ± 0.18 h, and 66.12 ± 46 L/h, respectively.

Glucose-lowering effect of metformin

The profile of change (%) of plasma glucose level for baseline (ΔPG) versus time is shown in Fig. 2. In the control group, the maximum PG was 18.76% at 4 h (sampling time just before meal), and returned to basal concentration at 6 h, and maintained thereafter until 10 h.

Table I. Pharmacokinetic parameters for metformin after 500 mg oral administration in healthy humans (n=22).

Parameter(unit)	Value
Non-compartmental analysis	
AUC (μg·h/mL)	7.64 ± 0.43
C_{max} (μg/mL)	1.42 ± 0.07
T_{max} (h)	2.59 ± 0.18
CL_t / F(L/h)	66.12 ± 46
Compartmental analysis	
V/F (L)	26.63
K_{el} (h^{-1})	0.35
K_a (h^{-1})	0.55
K_{cp} (h^{-1})	0.06
K_{pc} (h^{-1})	0.07
$t_{1/2\alpha}$ (h)	1.54
$t_{1/2\beta}$ (h)	12.01

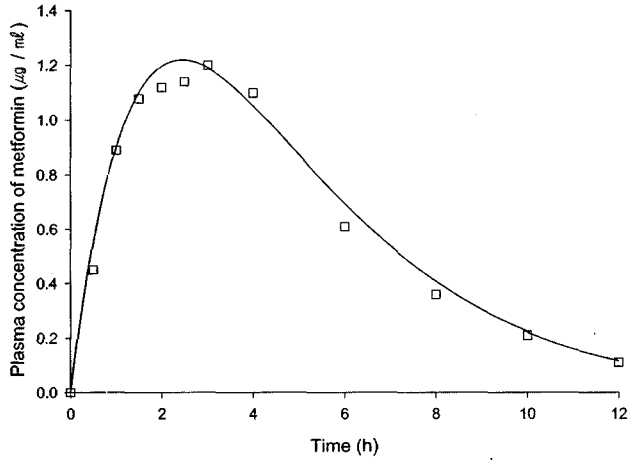

Fig. 1. Plasma concentration of metformin after a 500 mg administration in heathy human (n=22). Open squares are observed values and solid line is the fitted curve in oral two-compartment model by the weighted least squares (WLS) method using the ADAPT II program.

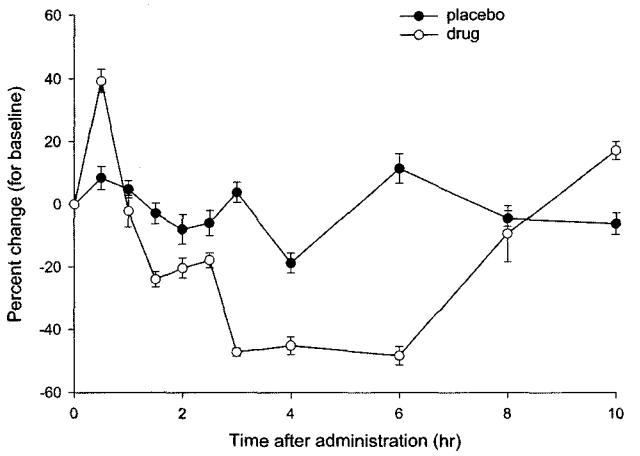

Fig. 2. Time course of percent change for baseline in plasma glucose concentration (mean ± S.E.M., n=11). The solid circles are control group (-●-) and the open circles are experimental group (-○-).

In the test group, metformin significantly inhibited the increase of glucose level. The glucose level was further decreased at 1.5 h, and maintained thereafter until 6 h after the administration. The maximum PG of metformin administration group was 48.3%. The glucose-lowering effect then rapidly returned to baseline within 8 h of dosing.

Table II. Pharmacodynamic parameter estimated by indirect model in ADAPT II program. Numbers in the parenthesis indicate the coefficient of variation for the parameter estimate.

Parameter	K_{in}	K_{out}	IC_{50}
	83.26 (11.07)	0.68	2.26 (35.73)

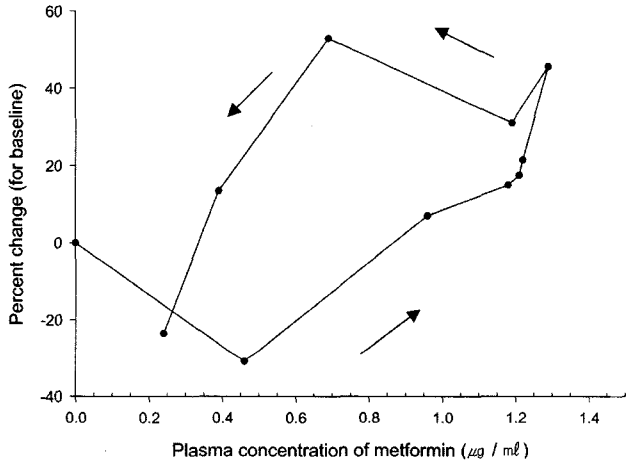

Fig. 3. Plot of metformin concentration versus change % for baseline in plasma glucose concentration. Plots are data from eleven healthy humans after a single oral dose of metformin 500 mg. The arrow indicates the time flow after metformin oral administration.

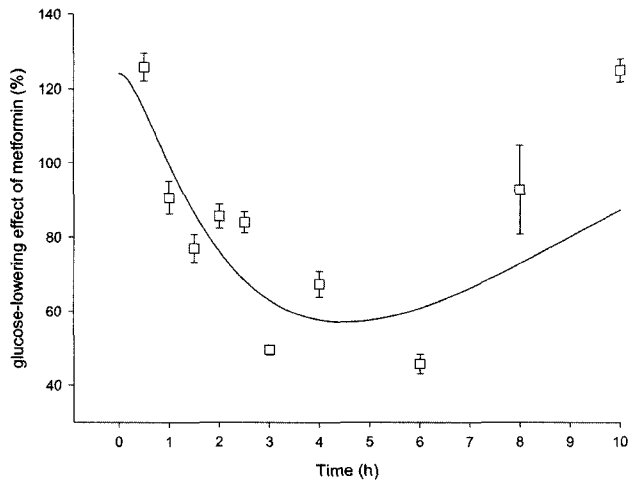

Fig. 4. Plots of plasma glucose lowering effect % and time course after a single dose 500 mg of metformin in volunteers (mean ± S.E.M, n=11). Data points are observed values. The solid line is the fitted curve from indirect response model with inhibition of the factors controlling the input of response.

Pharmacokinetic-pharmacodynamic modeling

The estimated pharmacodynamic parameters are listed in Table II. The mean concentration at half-maximum inhibition IC_{50}, K_{in}, and K_{out} were 2.26 μg/mL, 83.26 h^{-1}, and 0.68 h^{-1}, respectively.

Plots of plasma concentration of metformin against effect % of glucose lowering in time indicated a delay between the drug concentration change and the occurrence of effects. The metformin plasma concentration-response of glucose-lowering effect showed a counterclockwise hysteresis loop (Fig. 3). There was a significant difference between mean time of individual C_{max} (2.59 h) and mean time of individual maximum effect (6 h). Fig. 4 shows the average glucose-lowering effect versus time profile of 11 subjects of metformin. The curve means pharmacodynamic fit to measured effect values.

DISCUSSION

In this study, the glucose-lowering effect after oral dosing of metformin was analyzed as a function of plasma concentration. Although metformin, oral antihyperglycemic agent for type 2 diabetes mellitus, is clinically useful, the underlying mechanism has not been fully understood. However, the inhibition of the glucose synthesis and improvement of hepatic and peripheral tissue sensitivities to insulin is apparently involved for the action (McEvoy *et al.*, 2002). To our knowledge, pharmacokinetics-pharmacodynamic relationship has not been kinetically analyzed for metformin in the literature.

When the pharmacological effects are seen immediately and are directly related to the drug concentration, a pharmacodynamic model such as a linear model or a sigmoid E_{max} model is applied to characterize the relationship between drug concentration and effect. However, a number of drug responses may be considered indirect in nature. Aside from the distributional process, the observed delay between the plasma concentration and the pharmacological effect may also be explained by the underlying mechanism of the drug action (Walker *et al.*, 2003). Since metformin is considered to decrease the gluconeogenesis or glucose absorption thereby reducing the plasma glucose level, the elaboration of the observed response, may be secondary to a previous, time consuming synthesis or degradation of an endogenous substance. If the temporal dissociation between concentration and effect cannot be attributed to distributional process, mechanism-based indirect response models may be applicable. Indeed, indirect response models have been applied for numerous drugs, especially in cases where endogenous substances are involved in the expression of the observed response, similar to the case of metformin.

In our preliminary study, we attempted to analyze the

relationship using dirct response model. However, this line of approach was not successful as evidenced by poor correlation coefficients (i.e., the R^2 values less than 0.3) between the observed and calculate responses. In addition, the counterclockwise hysteresis loops observed in the metformin plasma concentration-glucose level changes indicate the presence of a time delay between the change in plasma concentration and the drug effects (Fig. 3). If a temporal dissociation between the time courses of concentration and effect exists, then the observed hysteresis in the concentration-effect relationship most likely results from a delay in pharmacokinetic distribution between central compartment and peripheral compartment, a postponement of cell signal for glycolysis and/or the process in the endogenous substances. The time delays between the plasma concentrations of metformin and the effect may be explained by postulating that the drug inhibits the gluconeogenesis in hepatocytes. The effect of metformin on glucose concentration was satisfactorily described by an indirect model with inhibition of input process. In the other class of drugs, histamine H_1-receptor antagonist such as mizolastine (Dayneka *et al.*, 1993), 5-HT_{1A} receptor agonist, flesinoxan have been applied to this indirect response model (Stepensky *et al.*, 2001).

In conclusion, using the indirect response model involving the inhibition of the input of the response, we demonstrated that the proposed model readily described the inhibitory effect of metformin on plasma glucose concentration. To our knowledge, this study represents the first application of pharmacokinetic-pharmacodynamic modeling for the glucose-lowering effect of metformin in humans. In addition, this is the first attempt to apply a mechanism-based indirect response model for the effect of meformin on plasma glucose level. Considering the fact that the response is clinically relevant response measures for the drug, our results may help elucidate the relationship between the glucose-lowering effect and the plasma concentration of metformin. Additional validation study of the model (e.g., applicability of the model in the case of multiple dosing situation) is under going in our laboratory. In addition, indirect response modeling strategy may be useful for drugs with a delay between the time courses for effect and drug concentration.

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