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 diabetes (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 $C_{\text{max}}$, $T_{\text{max}}$, $CL/F$ (apparent clearance), $V/F$ (apparent volume of distribution), and half-life of metformin were $1.42 \pm 0.07 \mu g/mL$, $2.59 \pm 0.18 h$, $66.12 \pm 4.6 L/h$, $26.63 L$, 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 $2.26 \mu g/mL$, $83.26 h^{-1}$, 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 sulfonylurea 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 the description of this relationship.
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 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 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 plasma 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, 4.6x250 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 :

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