한국임상약학회지 제26권 제4호
Korean J Clin Pharm, Vol. 26, No. 4, 2016
Original Article

Korean Journal of Clinical Pharmacy
Official Journal of
Korean College of Clinical Pharmacy
Available online at http://www.kccp.or.kr
pISSN: 1226-6051

건강한 한국인에서 미다졸람 집단약동학 분석: CYP3A 매개 약물상호작용 평가

신광희
경북대학교 약학대학
(2016년 7월 18일 접수 · 2016년 11월 1일 수정 · 2016년 11월 16일 승인)

Population Pharmacokinetics of Midazolam in Healthy Koreans: Effect of Cytochrome P450 3A-mediated Drug-drug Interaction

Kwang-Hee Shin
College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea
(Received July 18, 2016 · Revised November 1, 2016 · Accepted November 16, 2016)

ABSTRACT

Objective: Midazolam is mainly metabolized by cytochrome P450 (CYP) 3A. Inhibition or induction of CYP3A can affect the pharmacological activity of midazolam. The aims of this study were to develop a population pharmacokinetic (PK) model and evaluate the effect of CYP3A-mediated interactions among ketoconazole, rifampicin, and midazolam. **Methods:** Three-treatment, three-period, crossover study was conducted in 24 healthy male subjects. Each subject received 1 mg midazolam (control), 1 mg midazolam after pretreatment with 400 mg ketoconazole once daily for 4 days (CYP3A inhibition phase), and 2.5 mg midazolam after pretreatment with 600 mg rifampicin once daily for 10 days (CYP3A induction phase). The population PK analysis was performed using a nonlinear mixed effect model (NONMEM® 7.2) based on plasma midazolam concentrations. The PK model was developed, and the first-order conditional estimation with interaction was applied for the model run. A three-compartment model with first-order elimination described the PK. The influence of ketoconazole and rifampicin, CYP3A5 genotype, and demographic characteristics on PK parameters was examined. Goodness-of-fit (GOF) diagnostics and visual predictive checks, as well as bootstrap were used to evaluate the adequacy of the model fit and predictions. **Results:** Twenty-four subjects contributed to 900 midazolam concentrations. The final parameter estimates (% relative standard error, RSE) were as follows; clearance (CL), 31.8 L/h (6.0%); inter-compartmental clearance (Q) 2, 36.4 L/h (9.7%); Q3, 7.37 L/h (12.0%), volume of distribution (V) 1, 70.7 L (3.6%), V2, 32.9 L (8.8%); and V3, 44.4 L (6.7%). The midazolam CL decreased and increased to 32.5 and 199.9% in the inhibition and induction phases, respectively, compared to that in control phase. **Conclusion:** A PK model for midazolam co-treatment with ketoconazole and rifampicin was developed using data of healthy volunteers, and the subject's CYP3A status influenced the midazolam PK parameters. Therefore, a population PK model with enzyme-mediated drug interactions may be useful for quantitatively predicting PK alterations.

KEY WORDS: Midazolam, population pharmacokinetics, drug-drug interaction, CYP3A, healthy subjects

Pharmacokinetics (PK) is a powerful tool for understanding drug actions or describing the influence of drug-drug interactions. It is a useful method for evaluating the influence of drug-drug interaction on PK parameters, which eventually will alter the plasma drug levels with clinically significant consequences.[1] The PK for drug-drug interactions were often evaluated using non-compartmental analyses, which provide PK parameters without fitting the time-concentration profiles to any specific compartmental model.[1] However, the basic equations cannot be used to all drugs or detailed mechanisms such as first-pass metabolism or dug-interactions. Thus, complex mathematical models might be required to explain the profiles.[1] In such cases, compartmental PK analysis, which requires specific mathematical models, could be applied.

*Correspondence to: Kwang-Hee Shin, College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, 80 Daehakro, Bukgu, Daegu 41566, Republic of Korea
Tel: +82-53-950-8582, Fax: +82-53-950-8557
E-mail: kshin@knu.ac.kr

Midazolam, a benzodiazepine sedative agent, is almost exclusively metabolized by cytochrome P450 (CYP) 3A to 1'-OH-midazolam.[2] In the subsequent uridine diphosphate (UDP)-glucuronosyltransferase (UGT)-mediated phase II biotransformation, the main urinary metabolite, 1'-OH-midazolam-glucuronide is generated.[3] Furthermore, approximately 63-80% of the administered dose occurs in conjugated forms in urine within 24 h, while only 1% of the dose is excreted unchanged.[4] Owing to these metabolic characteristics, midazolam is used as the representative substrate for the in vivo assessment or evaluation of CYP3A activity as recommended by the European Medical Association (EMA) and the US Food and Drug Administration (FDA), and as a probe drug for CYP3A-mediated drug interaction studies.[5]

Similar to the application of midazolam, ketoconazole and rifampicin are used as representative CYP3A inhibitor and inducer, respectively[1] and are both drugs were also recommended by the EMA and FDA for use as representative interacting drugs for CYP3A.

This study was designed to establish a compartmental PK model for midazolam under CYP3A inhibition and induction status with ketoconazole and rifampicin co-administration, respectively. The population PK parameters of midazolam were estimated, and a feasible relationship between the PK parameters and various covariates including CYP3A5 genotypes was also investigated.

METHODS

Patients

The data used were obtained from 24 enrolled healthy Korean male subjects[6] who were aged 20 to 38 years and weighed 56.8 to 87.3 kg. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (SNUH). The clinical study was conducted at the Clinical Trials Center of SNUH (ClinicalTrials.gov: NCT01215214).

Drug administration and sample collection

This was an open-label, one-sequence, crossover study, divided into the three periods which consisted of midazolam PK evaluation, exploration of the midazolam PK changes influenced by ketoconazole, and assessment of the effects of rifampicin on midazolam PK (periods 1, 2, and 3, respectively).

On day 1, a single 1 mg intravenous dose of midazolam (Bukwang Pharmaceutical Co, Ltd., Seoul, Korea) was administered at approximately 9 a.m., and the subjects were discharged on the evening of day 2. From day 5 through 7, each subject visited the study center and received oral dose of ketoconazole (Spike®, Choong Wae Pharm, Seoul, Korea) 400 mg once a day at approximately 9 a.m. The subjects were readmitted on the evening of day 7 for the second administration of the study drug. On day 8, the subjects were administered intravenous dose of midazolam 1 mg 1 h after the pretreatment dose of ketoconazole. The subjects went through a modified blood sampling schedule in consideration of the increased exposure to midazolam and were then discharged on day 10. Each subject arrived in the morning and received rifampicin (Rifodex®, Chong Kun Dang Pharmaceutical Corp., Seoul, Korea) 600 mg orally once daily on day 13 through 21. All the subjects were hospitalized on the evening of day 20 for the third administration of midazolam intravenously.

On day 22, the subjects were administered rifampicin 600 mg and midazolam 2.5 mg concurrently, and the PK analysis of midazolam was repeated similar to the previous period.

The blood samples for the midazolam concentration determination were collected at the scheduled times during each period: prior to dosing (0 h), 10 min, 20 min, and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48* h (*period 2 only) after midazolam administration. The plasma, obtained by centrifugation at 2000 g, 4°C for 10 min, was immediately stored below −70°C until use in the analysis of midazolam.

The plasma concentrations of midazolam were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[6] The lower limit of quantification (LLOQ) for midazolam was 0.1 ng/mL. The standard calibration curves were linear over a concentration range of 0.3-100 ng/mL (coefficients of determination, $r^2 \geq 0.993$). The intra- and inter-day accuracy ranged from 96.6% to 99.6% and 99.4% to 102%, respectively, and the intra- and inter-batch precision, expressed as the coefficient of variation (CV, %), were less than 10%.

For the CYP3A5 genotyping, 1-mL blood samples were stored in cryotubes at −70°C. DNA was extracted from the blood samples using a QIAamp DNA blood mini kit (QIAGEN GmbH, Germany) and stored at −20°C. The genotypes were determined using the TaqMan Genotyping Assays (Applied Biosystems, Foster City, CA, USA) with a 7500 Real-Time polymerase chain reaction (PCR) System (Applied Biosystems, Foster City, CA, USA). The data was analyzed using the 7500 Real-Time PCR System software

(version 2.0.1, Applied Biosystems, Foster City, CA, USA).[6]

PK model building

A population PK model was constructed, and the concentration-time data were fitted to the compartmental models with nonlinear mixed effects regression techniques using NONMEM® (version 7.2, ICON Development Solutions, Ellicott City, MD, USA). The first order conditional estimation (FOCE) with interaction method was used.

Development of base population PK model

A three-compartment model with first-order elimination was exerted as the structural model. The IV midazolam administration, ketoconazole co-administration, and rifampicin co-administration phases were modeled simultaneously. An exponential model was applied to describe the inter-individual variability (IIV) of the midazolam PK parameters according to the following equation:

$$P_j = P_{pop} \times \exp(\eta_{IIV})$$

Where, P_{pop} is the mean value of the population parameters including the CL and the Vd, and η_{IIV} is a random variable representing the difference between individual (P_j) and population (P_{pop}) values that are normally distributed with a mean of 0 and variance of ω^2. The additive, proportional, and combined error model were investigated for the residual variability using these equations:

Additive error model: $C_{ij}=C_{pred,\ ij}+\varepsilon_{add,\ ij}$
Proportional error model: $C_{ij}=C_{pred,\ ij} * (1+\varepsilon_{pro,\ ij})$
Combined error model: $C_{ij}=C_{pred,\ ij} * (1+\varepsilon_{pro,\ ij})+\varepsilon_{add,\ ij}$

where, C_{ij} is the observed concentration; $C_{pred,\ ij}$ is the i_{th} concentration predicted based on the parameters and study scheme in the j_{th} patient; and ε is the normally distributed residual error with a mean of 0 and variance of σ^2.[7,8]

Covariate model

Covariates including the categorical covariates (CYP3A5 genotypes) and continuous covariates (age, body weight, and height) were sequentially added to CL and Vd. None of the covariates influenced the midazolam CL or Vd. The final model was selected using the objective function value (OFV), visual exploration of the distribution of observed and predicted values, goodness-of-fit plots, and physiological relevance. A drop in the OFV by more than 3.84 represents improvement in model fit with a statistical significance ($p < 0.05$).[9]

PK model evaluation

The adequacy and performance of the developed population PK model were evaluated using a visual predictive check. We simulated 200 data sets using the final PK model. The model adequacy was assessed by comparing the median and the 5th and 95th percentiles calculated from simulated concentrations with measured concentrations.

A nonparametric bootstrap analysis was performed to validate the reliability and stability of the model developed. The 95% confidence intervals (CIs) were calculated for each PK parameter using the 2.5th, and 97.5th percentiles of 2000 bootstrap estimates and the final PK parameters were subsequently compared with bootstrap estimates.

RESULTS

Demographics

The demographics of the subjects are summarized in Table 1, which shows there were 24 male subjects in the midazolam model building dataset. The enrolled subjects were aged 20 to 38 years and weighed 56.8 to 87.3 kg. The model building population revealed that the minor allele frequency of CYP3A5*3 A6956G was 0.792.

Model development

The plasma midazolam concentration-time curves were well explained using a three-compartment model (Fig. 1). Because the CL of midazolam differed among the three phases, control, inhibition, and induction, we included the treatment factor while estimating CL. The addition of TRT (treatment) as a

Table 1. Summary of demographics of subjects.

		modeling population mean [range]
number of subjects*		24
age (years)		26.5 [20-38]
body weight (kg)		69.2 [56.8-87.3]
CYP3A5 genotyping*		
A6956G	A/A	0
	A/G	10
	G/G	14

*number of subjects

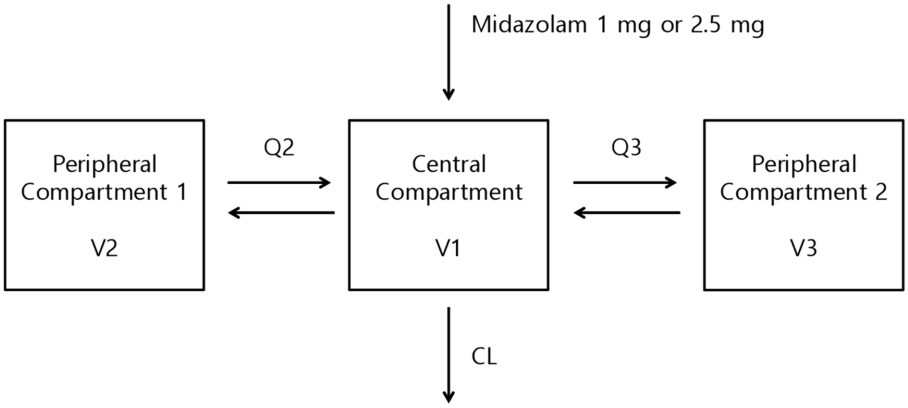

Fig. 1. Final model structure.

covariate for CL enhanced the fit of the data. Otherwise, the CYP3A5*3 genotype was not selected as a final covariate. With the generalized additive model (GAM) analysis, none of the other covariates was selected as parameters for the CL or Vd. Table 2 presents the parameter estimates of the final model.

Model validation

Fig. 2 describes the diagnostic goodness-of-fit plots for the final model. The visual predictive checks for the final model with the full data set of 24 subjects are shown in Fig. 3. The majority of the observations were located within the prediction interval, and 1993 runs were successfully minimized during the bootstrap resampling analysis. The median values of the bootstrap procedure were comparable with the parameter estimates obtained using the modeling procedure.

DISCUSSION

The current study was designed to develop a population PK model of midazolam in healthy male Korean subjects. The PK samples were collected according to a serial schedule after dosing to ensure a precise estimate of the PK parameters. CYP3A5 genotyping was conducted to investigate the effects of genetic polymorphisms on the CL of midazolam. The final CL model suggests that midazolam CL decreased and increased to 32.5 and 199.9% in the inhibition and induction phases, respectively, compared to that in the control phase.

Similar to the previous midazolam study results, the time-concentration profiles of midazolam in this study were more fitted to a three-compartment model that they were to a two- or one-compartment model. The basic structures have been selected as three-compartment models in several studies

Table 2. Population parameter estimates for the final model.

parameter		final model		bootstrap (n=2000)	
		estimates	relative standard error (%)	median	95% confidence interval
θ_1	CL (L/h)	31.8	6.0	31.6	28.6-35.8
θ_2	effect of ketoconazole	0.675	2.0	0.675	0.649-0.859
θ_3	effect of rifampicin	1.0	7.9	1.0	0.701-1.141
θ_4	V_1 (L)	70.7	3.6	70.8	66.8-75.4
θ_5	Q_2 (L/h)	36.4	9.7	36.1	29.7-44.8
θ_6	V_2 (L)	32.9	8.8	32.9	27.1-38.6
θ_7	Q_3 (L/h)	7.37	12.0	7.30	5.73-9.65
θ_8	V_3 (L)	44.4	6.7	44.8	36.5-51.2
ω^2_{CL}	inter-individual variability	0.028	36.8	0.025	0.011-0.045
ε^2	residual variability	1	-	1	-

Clearance, $CL=\theta_1*(1-TRT_1*\theta_2)*(1+TRT_2*\theta_3)*\exp(\omega^2 CL)$; for ketoconazole treatment, $TRT_1=1$, otherwise $TRT_1=0$; for rifampicin treatment, $TRT_2=1$, otherwise $TRT_2=0$; Q, inter-compartmental clearance; V, volume of distribution.

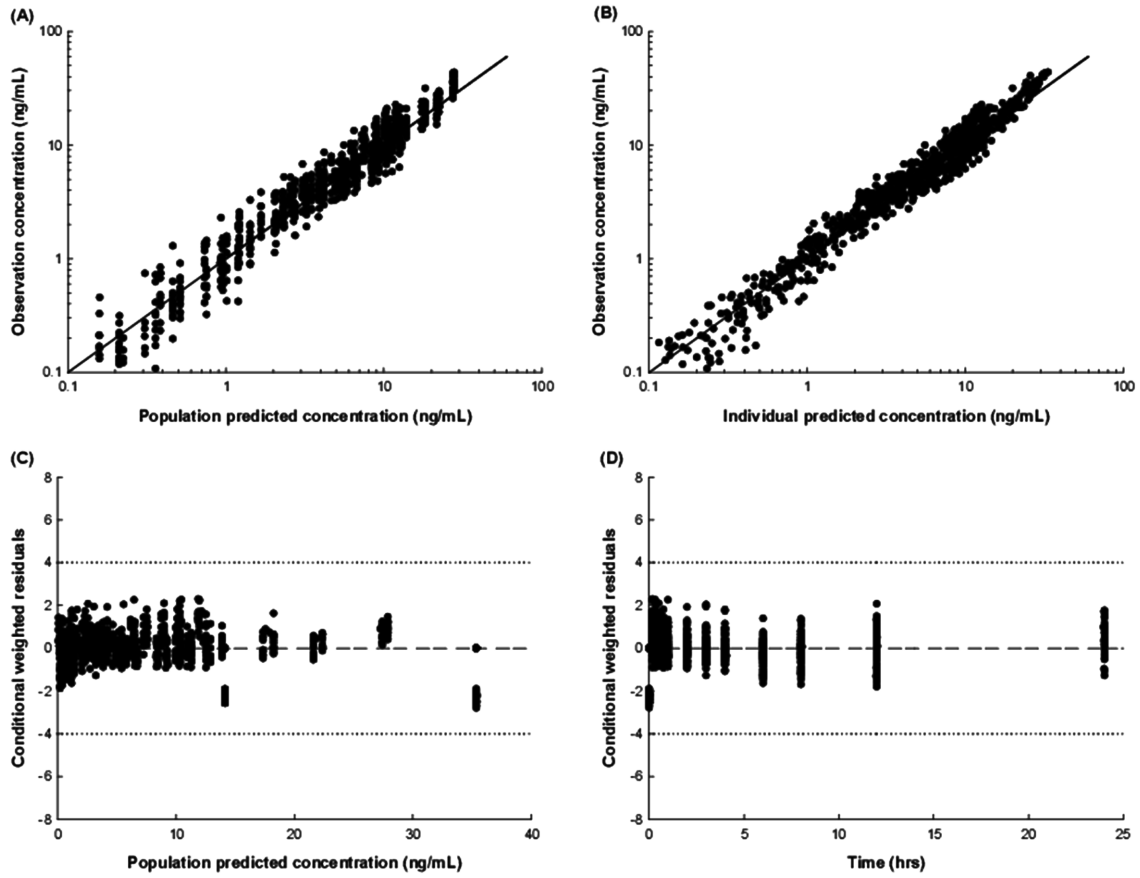

Fig. 2. Basic model diagnosis plot constructed using final pharmacokinetic (PK) model (A) Observations (DV) vs. population predictions (PRED) and (B) DV vs. individual predictions (IPRED). (C) Individual weighted residuals (IWRES) vs. IPRED and (D) conditional weighted residuals (CWRES) vs. time (TIME).

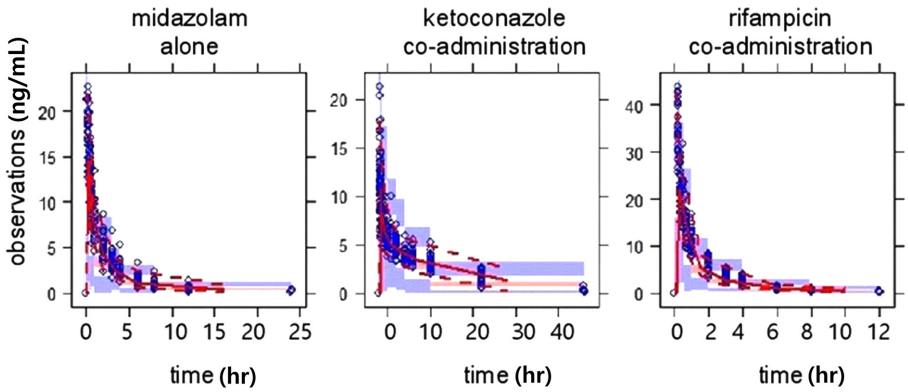

Fig. 3. Visual predictive check (VPC) of midazolam plasma concentration from final model Solid red line represents median observed plasma concentration and dashed red lines represents 5th and 95th percentiles of model simulation. Red and blue shaded area depicts simulation-based 95% confidence intervals (CIs) for the median and corresponding model predicted 5th and 95th percentiles, respectively. Observed plasma concentrations are shown as blue dots.

including midazolam drug interaction study.[10-12)]

The description of the detailed mechanistic interactions was limited because the time-concentration profiles of ketoconazole and rifampicin were not determined in the current study. The concentration data of both affector drugs was obtained, which enabled the assessment of time course of interaction or related

parameters. Moreover, the PK of midazolam after IV administration was assessed and the interactions of the orally administered midazolam with ketoconazole or rifampicin should have been considered separately. Despite these limitations, this study provides the compartmental PK analysis of the most representative CYP3A substrate, midazolam, with or without a representative inhibitor or inducer. In addition, the impact of genetic polymorphism of CYP3A5 was assessed as a covariate.

In conclusion, in the present study, a population PK model of midazolam was developed under CYP3A inhibition and induction status in healthy male Korean subjects. The model described the influence of two drugs simultaneously. The midazolam CL decreased and increased to 32.5 and 199.9% with ketoconazole and rifampicin co-administrations (CYP3A inhibition and induction), respectively. Therefore, the population PK model with the enzyme-mediated drug-interaction may be useful for quantitatively predicting the PK alterations.

CONFLICT OF INTEREST STATEMENT

The author does not have any conflicts of interest to declare regarding this article.

ACKNOWLEDGEMENTS

The clinical trial was performed at the Seoul National University Hospital Clinical Trials Center, and the determination of midazolam concentration was conducted in the Department of Clinical Pharmacology & Therapeutics, Seoul National University College of Medicine and Hospital. I appreciate Dr. Joo-Youn Cho and staffs of Department of Clinical Pharmacology & Therapeutics, Seoul National University & Hospital for performing the clinical study and quantifying the bio-analysis. This research study was supported by the Kyunpook National University Research Fund, 2013. Finally, Jihye You participated in the compartmental PK analysis.

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