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Population pharmacokinetics and inter-laboratory variability of sildenafil and its metabolite after oral administration in Korean healthy male volunteers

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This study was to clarify population pharmacokinetics (PK) of sildenafil and its metabolite, N-desmethyl sildenafil (NDS) in Korean healthy male population using a pooled data from multiple clinical trials in consideration of inter-institution and inter-laboratory difference. A population PK analysis was performed with data of 243 healthy volunteers from five single-center (4 centers) comparative PK trials. The dataset included 7,376 sildenafil and NDS concentration (3,688 for each analyte) observed during 24 hours after the single dose of original sildenafil (either 50 mg or 100 mg of Viagra®). The plasma concentration was assayed in two laboratories. Various model structure was tested and the final model was evaluated using visual predictive checks. Demographic and clinical variables were assessed as potential covariates for PK parameters. A one-compartment first-order elimination model with proportional error was selected for the dispositional characteristics of sildenafil, and two-compartment model was chosen for NDS. Three transit compartments with Erlang-type absorption for fast absorption pathway and one compartment for slow absorption pathway constructed overall absorption model. The first-pass effect was rejected since it does not improve the model. The difference of NDS level by the bioanalysis laboratory was selected as the only covariate. Even though a direct comparison was difficult, the general trend in PK of sildenafil and NDS for Korean healthy male was considered similar to that of the other populations reported previously. It is recommended that the laboratory effect should be explored and evaluated when dataset is built using results from several laboratories.

ORIGINAL ARTICLE

Introduction

The prevalence of erectile dysfunction (ED) varies between 18 and 52% depending on the study, and tends to increase with age.[1-4] The mechanism of normal penile erection involves nitric oxide (NO) release and the upregulation of cyclic guanosine monophosphate (cGMP), which results in the relaxation of penile smooth muscles and the regulation of intracellular calcium in the corpora cavernosa.[5,6] Malfunctions in this mechanism manifest as a reduction in NO release, leading to smooth muscle contraction and, eventually, to ED.[7] ED etiology includes organic and psychogenic mechanisms,[5] and organic etiology can be explained by malfunctions of the corpus cavernosum, which is controlled by NO release and cGMP stimulation.[7,8]

Even though ED is a major cause of sexual dysfunction in males,[3] effective oral therapy for ED was not available until 1998, when Sildenafil was first introduced by Pfizer.[5,9] Sildenafil is a first-in-class agent for ED oral therapy, and is a novel selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5). Sildenafil acts on PDE5, which is abundant in vascu-

lar smooth muscle, and blocks conversion of cGMP to GMP, which leads to the accumulation of calcium ions and, finally, to smooth muscle relaxation.[10] The pharmacokinetic (PK) characteristics[11,12] and interactions[13-15] of sildenafil as the original film-coated tablet (FCT) are well established (in Caucasian), and the metabolism of sildenafil mainly occurs in the liver by cytochrome P450 3A4.[8,16] Five metabolites of sildenafil have been reported to date,[11] the majority of which are N-desmethyl sildenafil (NDS, UK-103,320),[17] which is the primary metabolite analyzed in this investigation.

When the original FCT patent expired, a number of clinical trials were conducted which involved newer formulations including orally disintegrating film,[18] and the PK characteristics were compared between formulations. By pooling the data of the FCT from such trials, we were to build a mixed-effects PK model of sildenafil and its metabolite in healthy Korean male population. Because the data were generated from different clinical trial centers (CTC, site) of which the samples were assayed in different laboratories for plasma concentration measurement, the site effect and laboratory effect were also evaluated during the model development process.

Methods

Ethical considerations

This study is a retrospective pharmacometric analysis that uses de-identified data from clinical trial subjects. The study protocols were approved by the respective institutional review boards in accordance with the ethical standards for studies in humans established by the Declaration of Helsinki and its amendments, Good Clinical Practice of the International Conference on Harmonization, and Korean laws and regulations when the studies were first performed. Written informed consent was obtained from all the volunteers before enrollment in each study.

Dataset

Data were obtained from five different randomized, open-label, single-dose, two-way crossover comparative PK studies (Protocol 1~5), conducted at four CTCs in the Republic of Korea. All subjects included in the dataset were healthy Korean males who met study-specific eligibility criteria (N = 243). The inclusion and exclusion criteria varied between study protocols; however, all required the subjects to be healthy. Common inclusion criteria were: Age ≥ 20 years; Body weight ≥ 50 kg and within ±20% of individual ideal body weight.

In each study, for the period of the reference (original) formulation, a single tablet of reference formulation (Viagra® 50 mg tablet for Protocol 1, 2, 4, 5; Viagra® 100 mg tablet for Protocol 3; both manufactured by Pfizer) was given to each subject after more than 10 hours of fasting. At least 15 samples were collected within 24 hours after dosing in each study. Whole blood samples obtained from all subjects were preserved in the refrigerator at -70°C before analysis. The samples were analyzed for sildenafil and NDS concentration.

In total, 7,376 concentrations were included in the dataset. In the elimination phase (12~24 h), 280 observation points were below quantifiable limit (BQL). The proportions of BQL data in this period were 40.9% for sildenafil and 16.7% for NDS. No observed value was excluded as an outlier. For protocols 1, 4, and 5, the analyses were performed in laboratory X, while analyses were performed in laboratory Y for protocols 2 and 3. Study-specific sample sizes, basic subject demographics, dosage, PK sampling scheme, and the laboratory where the samples were analyzed are summarized in Table 1.

Bioanalysis

Sildenafil and NDS concentrations were evaluated using high-performance liquid chromatography coupled with mass spectrometry. Each analysis laboratory followed an identical peer-reviewed bioanalysis article;[19] however, the protocol was independently set and conducted by each laboratory. Thus, the analysis procedure details, including the volumes of specific solution added in the analytic procedures were not perfectly identical, and the lower limit of quantification (LLOQ) were not consistent between laboratories. The LLOQs were 2 ng/ml and 1 ng/ml in Laboratory X for sildenafil and NDS, respectively, and

Table 1. The five clinical trial protocols and their properties

Protocol No.	Trial site	Sample size	Bioanalysis laboratory	Dose (mg)	PK sampling time (hr) (total number of samples)	Age (years) Mean(SD)	Weight (Kg) Mean(SD)
1	A	50	X	50	0, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, 24 (17)	25.18(2.94)	67.55(7.09)
2	B	48	Y	50	0, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.33, 1.67, 2, 3, 4, 8, 12, 24 (15)	23.38(2.52)	69.47(7.59)
3	C	52	Y	100	0, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 (15)	25.48(3.35)	68.68(6.41)
4	D	45	X	50	0, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, 24 (17)	23.78(2.8)	69.53(7.94)
5	D	48	X	50	0, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, 24 (17)	22.67(1.21)	64.93(7.25)

1 ng/ml and 0.5 ng/ml in Laboratory Y, respectively.

Population PK analysis

Mixed-effects modeling analysis was performed using NONMEM (version 7.3, Icon Development Solution, Ellicott City, MD). The first-order conditional estimation with interaction (FOCE-I) was applied whenever possible. The between-subject variability (BSV) of the PK parameter was applied exponentially:

$$P_{ij} = \theta_j \cdot \exp(\eta_{ij})$$

where P_{ij} is the j-th parameter estimate for the i-th individual, θ_j is the typical population value of the j-th parameter, and η_{ij} is a random variable normally distributed with a mean of 0 and variance of ω_i^2. Various compartment models were tested to determine the best distributional structure of sildenafil, and first-order kinetics was assumed for all PK processes except drug absorption. The model was diagnosed based on both numerical and visual criteria such as objective function value (OFV) and diagnostic plots, including goodness-of-fit and individual plots. In each modeling step, model improvement was checked with a likelihood ratio test, and a better model was selected when the OFV decreased more than 3.84 ($p < 0.05$, $df = 1$) or 5.99 ($p < 0.05$, $df = 2$) by the addition of a parameter(s).

During the covariate model-building process, stepwise forward selection and backward elimination were applied. The potential covariates were age, body weight, aspartate aminotransferase/alanine aminotransferase level at screening, serum creatinine, blood urea nitrogen, creatinine clearance (CLCR), site and laboratory effects. The CLCR was calculated from the Cockcroft-Gault equation. Various forms of covariate models were tested, including linear, piecewise, power, and exponential equations for any of the continuous or categorical covariates. The covariate screening process was performed using visual (parameter versus variable scatterplots) and numerical (generalized additive modeling implemented by Xpose (version 4.5.0) approaches. In the forward selection of covariates, variables that decreased the OFV by > 3.84 ($P < 0.05$) and decreased the inter-individual variabilities were selected. Covariates that did not increase the OFV by > 6.63 ($P < 0.01$) in backward elimination were removed from the model.

Because the frequency of BQL data was rather higher in the elimination phase of sildenafil, a likelihood-based approach, so-called, M3 method was utilized since it was the gold-standard for the handling of BQL data.[20] Because of this methodological aspect for which the residual-based diagnostics were not suitable, the final model was evaluated using visual predictive check (VPC). In the VPC procedures, a simulation of 1,000 replicate population was performed with the identical subject composition to the original dataset and the raw data were overlaid on the 90% prediction interval.

Results

A one-compartment first-order elimination model with proportional error was selected for the dispositional characteristics of sildenafil while a two-compartment model was chosen for NDS. Because the exact amount of NDS produced from sildenafil cannot be assessed, the metabolic clearance of sildenafil to NDS was assumed to be a half of total clearance ($k_{20} = k_{24}$) based on an in vitro metabolism test.[21] All drug movement between compartments was assumed to follow a first-order process and it was considered acceptable in the final model fit. The outline of final model was described on Figure 1.

During the modeling procedure, the incorporation of first-pass effect, which describes a direct movement from the depot compartment to the observation compartment of NDS, was tested; however, it showed insufficient model improvement ($\Delta OFV < 3.84$, $X^2_{a=0.05,\ \upsilon=1}$) so it was excluded in the final model. Without the first-pass effect, the absorption of sildenafil was described using two distinct absorption pathway. Three transit compartments were recruited using Erlang-type modeling for the rapid pathway and one compartment was added for the slow pathway. Because the single pathway model substantially under-predicted concentrations after the peaks, dual absorption pathways were selected, that improved the overall time-concentration profile. The first-order absorption rate constant for rapid ($K_{a,1}$) and slow ($K_{a,2}$) pathways were 9.38 hr^{-1} and 0.119 hr^{-1}. The sildenafil concentration variability was mainly explained by the ω^2 estimate for $K_{a,1}$, which was 59.3% of the coefficient of variation.

The only covariate selected was the effect of laboratory difference on NDS concentration level ($\Delta OFV = -40.079$). This was a general trend, regardless of the observation time, so it was reflected in the scaling factor for NDS concentration ($S4 = V4 \cdot (1 + LVF * \theta) / 1000$, where LVF is 0 for observations analyzed at laboratory X and 1 for laboratory Y). Judging from the final es-

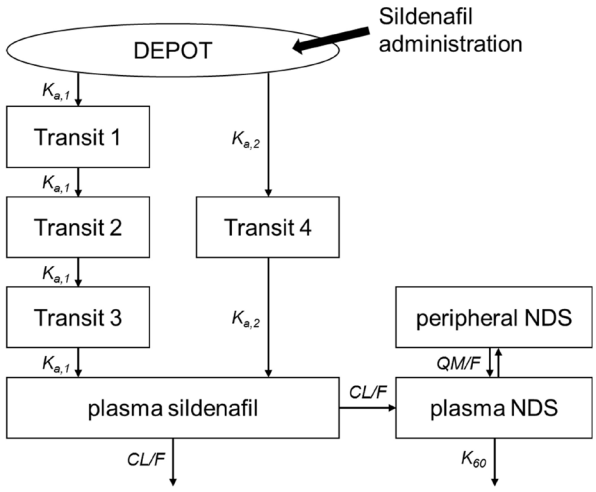

Figure 1. Outline of the final model.

Table 2. Final sildenafil and NDS parameter estimates

Parameters	Description	Estimates	% RSE[a]
Fixed effects			
$K_{a,1}$ (h^{-1})	Rate constant of rapid absorption	9.38	1.663
$K_{a,2}$ (h^{-1})	Rate constant of slow absorption	0.119	1.118
CL/F (L/h)	Apparent clearance of sildenafil	37.1	2.106
V5/F (L)	Apparent volume of sildenafil central compartment	174	1.644
V6/F (L)	Apparent volume of NDS central compartment	14.2	5.282
K60 (h^{-1})	Elimination rate constant of NDS	11.3	3.531
V7/F (L)	Apparent volume of NDS peripheral compartment	444	7.658
QM/F (L/h)	Inter-compartmental clearance of NDS	40.9	5.515
LVF	Inter-laboratory variability factor on NDS concentration	-0.347	-2.634
Random effects			
$\omega_{Ka,1}$	Between-subject variability of $K_{a,1}$	0.301	9.701
$\omega_{Ka,2}$	Between-subject variability of $K_{a,2}$	NE	-
$\omega_{CL/F}$	Between-subject variability of *CL/F*	0.137	12.993
$\omega_{V5/F}$	Between-subject variability of *V5/F*	0.137	13.645
$\omega_{V6/F}$	Between-subject variability of *V6/F*	0.194	7.01
ω_{k60}	Between-subject variability of *K60*	NE	-
$\omega_{V7/F}$	Between-subject variability of *V7/F*	0.0369	98.92
$\omega_{QM/F}$	Between-subject variability of *QM/F*	0.184	21.63
Residual errorb			
$\sigma_{additive,1}$	Additive error for sildenafil	NE	-
$\sigma_{prop,1}$	Proportional error for sildenafil	0.441	-
$\sigma_{additive,2}$	Additive error for NDS	NE	-
$\sigma_{prop,2}$	Proportional error for NDS	0.515	1.464

[a] %RSE, relative standard error; [b] σ_{prop}, proportional error; NDS, N-desmethyl sildenafil; NE, Not estimated.

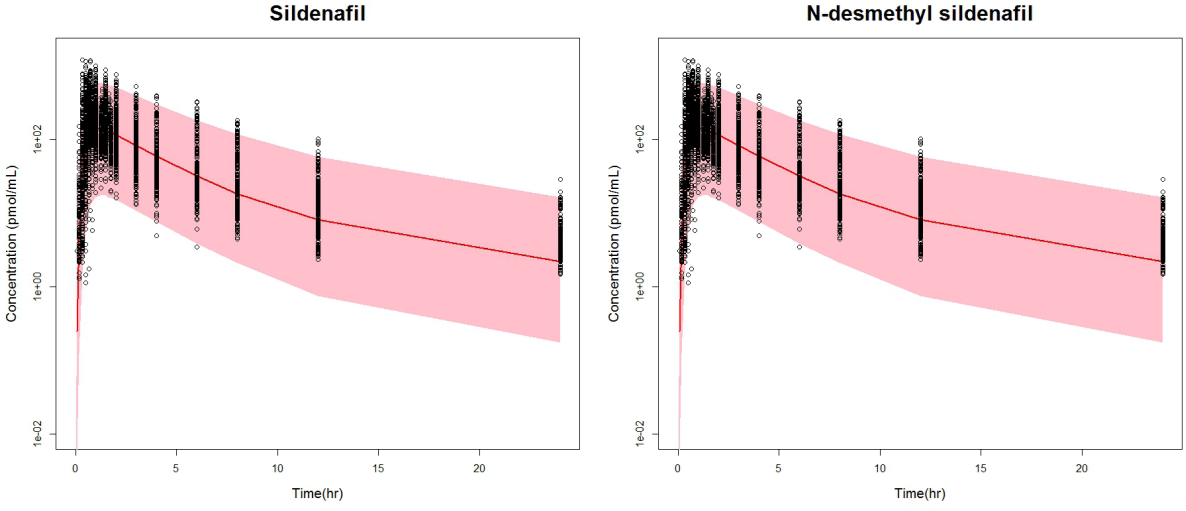

Figure 2. Visual predictive check. Solid line: prediction median, Colored band: 90% prediction interval.

timate of corresponding θ, laboratory Y produced 53.1% higher values than laboratory X for NDS levels. The final parameter estimates are summarized in Table 2. The visual predictive check plots are shown in Figure 2.

Discussion

Data from five single-center comparative PK trials were merged and analyzed together to characterize the PK properties of sildenafil and NDS in Korean healthy male subjects when given as the original sildenafil formulations. The basic assumption that population characteristics are homogeneous across the trials seemed acceptable judging from similar inclusion/exclusion criteria and distributions of subject weight and age. In addition, demographic variables or the site effect were not selected as significant covariate for any PK parameter. There could be some minor discrepancies in subject characteristics between trials according to inclusion/exclusion criteria; however, these were considered negligible because the subjects' medical conditions basically satisfied the requirements as healthy subjects and most lifestyle factors which might be influential to PK were generally limited in those trials. Based on this homogeneity, we could also investigate whether there is any formulation effect (50 mg versus 100 mg tablet), site effect and/or laboratory effect.

The model developed in this study incorporates the PKs of sildenafil and its metabolite NDS linked with unidirectional first-order metabolic rate constant which indicates irreversible biotransformation of sildenafil to NDS. Biochemically, sildenafil does not show any transformation from metabolite to parent *in vivo*,[11] so the developed model may be considered acceptable. In addition, the omission of first-pass effect based on the significant model improvement criteria seemed consistent to the findings from the dataset where it was shown that the increase in NDS concentration after dosing was delayed compared to that in sildenafil. One of the previous reports have shown a first-pass effect for sildenafil;[22] however, the reason for the disagreement in this study cannot be assessed. Judging from the finding that dose effect was not meaningful for any PK parameter, the dose-proportionality of Viagra® 50 mg tablet and Viagra® 100 mg tablet could be ensured, which is consistent with previous report.[12]

Population PK analysis of sildenafil on ED patients[23] was derived from five phase III studies, and the sildenafil-only model was also described with one-compartment; however, the absorption characteristics were fitted with only one first-order absorption rate constant. From the analysis, the authors derived a number of covariates that could be incorporated into the model while only LABD was found in this study. The reason of this discrepancies was considered to be the homogeneity of population characteristics in this study. Despite the differences, the overall concentration-time profile of sildenafil observed in this study was similar to those reported elsewhere.

PK analysis revealed inter-laboratory variability between the two laboratories, specifically for NDS. The laboratory factor attached to the scaling factor successfully acknowledged OFV reduction, which means that the laboratory factor contributed significantly to the difference in NDS level over the observation period. This implied that, even though similar LC/MS/MS assay procedure was utilized, the concentration results may vary according to the miscellaneous differences between laboratories. The comparison between results from one laboratory may not be problematic while care should be taken for a comparison of outputs from two or more laboratories.

We could conclude that the PK characteristics of sildenafil and NDS in the Korean population included in this study were relatively homogeneous with no fixed effect influential to PK and were similar to those reported previously.[23] The inter-laboratory differences in the analyzed concentration level may be a significant factor for PK analyses similar to this study, so that the laboratory effect should always be explored and evaluated when dataset is built using results from several laboratories.

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Conflict of interest

The authors have declared that no conflicts of interest exist regarding this study.

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