The Effects of Ketoconazole and Rifampicin on the Pharmacokinetics of Mirodenafil in Healthy Korean Male Volunteers: An Open-Label, One-Sequence, Three-Period, Three-Treatment Crossover Study

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ABSTRACT

Background: Mirodenafil, a phosphodiesterase 5 inhibitor reported to be effective in the treatment of erectile dysfunction, is metabolized by cytochrome P450 (CYP) 3A4 to the active metabolite *N*-dehydroxyethyl mirodenafil. Mirodenafil may have drug–drug interactions with ketoconazole and/or rifampicin.

Objective: The aim of this study was to investigate the effects of a potent inhibitor (ketoconazole) and inducer (rifampicin) of the CYP3A4 isozyme on the pharmacokinetics of mirodenafil to meet the regulatory requirements for the marketing of mirodenafil in Korea.

Methods: An open-label, 1-sequence, 3-period, 3-treatment crossover study was conducted over 22 days in healthy Korean male volunteers. Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days (period 3). Serial blood samples were collected for pharmacokinetic analysis after the administration of mirodenafil in each study period. Plasma concentration–time data for mirodenafil and its major metabolite, *N*-dehydroxyethyl mirodenafil, were determined using LC-MS/MS and analyzed by a noncompartmental method. The results for mirodenafil coadministration with either ketoconazole or rifampicin were compared with those for mirodenafil alone. Adverse events (AEs) were identified by asking general health-related questions of the subjects, by physical examination, and by subject self-report throughout the study period.

Results: Nineteen subjects were enrolled (mean [SD] age, 23.2 [2.76] years [range, 19–29 years]; weight, 69.3 [6.50] kg [range, 61.0–84.0 kg]; body mass index, 22.4 [1.77] kg/m^2 [range, 20.0–26.0 kg/m^2]) and 18 subjects completed the study. One subject discontinued the study due to protocol violation and was replaced. The $AUC_{0-\infty}$ of mirodenafil increased 5.04-fold (90% CI, 3.78–6.72) and the metabolic ratio decreased 0.21-fold after pretreatment with ketoconazole compared with mirodenafil alone. After pretreatment with rifampicin, the $AUC_{0-\infty}$ of mirodenafil decreased 0.03-fold (90% CI, 0.02–0.05) and the metabolic ratio increased 2.9-fold. Twelve cases of headache, 6 of nasal congestion, 2 of feeling hot, 2 of epistaxis, and 1 each of dizziness, nausea, and somnolence were considered to be related to administration of mirodenafil. Twenty-eight AEs were reported in period 2 (in 68.4% of subjects), during which systemic exposure to mirodenafil was highest, whereas 7 AEs were reported in period 1 (in 31.6% of subjects) and 5 AEs in period 3 (in 16.7% of subjects).

Conclusion: In these healthy Korean male volunteers, the coadministration of ketoconazole and rifampicin resulted in significant changes in systemic exposure to mirodenafil. (*Clin Ther.* 2009;31:3009–3020) © 2009 Excerpta Medica Inc.

Key words: mirodenafil, drug–drug interaction, CYP3A4, ketoconazole, rifampicin.

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INTRODUCTION

Mirodenafil* is a potent, selective, oral phosphodiesterase type 5 (PDE5) inhibitor reported to be effective in the treatment of erectile dysfunction; it was approved by the Korean Food and Drug Administration in 2007.[1–3] Mirodenafil was reported to be well tolerated at doses up to 200 mg; was absorbed rapidly, with C_{max} reached 1.25 hours after dosing; and was eliminated with a $t_{1/2}$ of 2.5 hours in a Phase I clinical study in healthy volunteers.[2] The drug was reported to be effective in improving erectile function in 223 Korean male subjects with broad-spectrum erectile dysfunction in a Phase III clinical trial.

An in vitro study of human liver microsomes and complementary DNA-expressed cytochrome P450 (CYP) found that mirodenafil is predominantly metabolized to its active metabolite, *N*-dehydroxyethyl mirodenafil, by CYP3A4 and to a lesser extent by CYP2C8; the intrinsic clearance for metabolism to the metabolite by CYP3A4 was 3.3- and 44.4-fold higher than by CYP2D6 and CYP2C8, respectively.[4] These results suggest that the pharmacokinetics of mirodenafil may be affected by the coadministration of CYP3A4 inhibitors such as ketoconazole, erythromycin, and cimetidine, or by CYP3A4 inducers such as rifampicin and barbiturates.[5] Ketoconazole, an imidazole antifungal agent, is a potent inhibitor of the CYP3A4 isozyme.[6] Rifampicin, a bactericidal antibiotic drug, is a potent inducer of the CYP3A4 isozyme and has been found to reduce the plasma concentrations of several CYP3A4 substrates.[7]

In a previous study, the oral bioavailability of mirodenafil was estimated to be 24.1% to 43.4% for doses of 10 to 40 mg/kg in rats, and this low bioavailability was reported to be mainly due to the first-pass effect.[8] Mirodenafil is primarily cleared to *N*-dehydroxyethyl mirodenafil by intestinal and hepatic metabolisms that include CYP3A4.[4] Thus, CYP3A4 is considered to be a major cause of the low bioavailability of mirodenafil. Because CYP3A4 is the most abundant isozyme of the CYP system in the adult human liver,[9] the possible drug–drug interactions of mirodenafil with CYP3A4 substrate agents may have an important effect on clinical use.

This study investigated the pharmacokinetic profile of mirodenafil after pretreatment with ketoconazole and rifampicin in healthy Korean male volunteers to meet the regulatory requirements for the marketing of mirodenafil in Korea.

*Trademark: Mvix® (SK Chemicals Co., Ltd., Seoul, Korea).

SUBJECTS AND METHODS

Subjects

Healthy Korean male volunteers aged 19 to 50 years were eligible for inclusion in this study, provided they weighed >55 kg and were within 20% of their ideal weight. Subjects were excluded from the study if they exhibited any clinically significant disease or had an abnormal laboratory test result in clinical biochemistry, hematology, or urinalysis.

Study subjects were prohibited from consuming caffeinated and alcoholic beverages, smoking, and doing strenuous exercise for 3 days before study drug administration and until the end of the last study period. Subjects were hospitalized for 12 hours before receipt of the mirodenafil dose and for 24 hours after dosing. All subjects were maintained in a fasting state until 4 hours after the mirodenafil administration.

Study Design and Treatment

This was an open-label, 1-sequence, 3-period, 3-treatment crossover study. Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days, with a single dose of mirodenafil given after the third ketoconazole administration (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days, with a single dose of mirodenafil given on the day after the last rifampicin administration (period 3) (**Table I**).

Tolerability was assessed by investigator monitoring for adverse events (AEs), physical examinations, measurement of vital signs, 12-lead ECGs, and clinical laboratory tests. Physical examinations and ECGs were performed at predefined intervals throughout the study. Vital signs and heart rate were measured with the subject in a sitting position after a 3-minute rest using an automated device (Solar 8000M Patient Monitor, GE Healthcare, Waukesha, Wisconsin) and body temperature was measured with a tympanic thermometer. Blood samples were drawn for clinical laboratory assessments of hematology and blood chemistry, and urinalysis was conducted.

The study protocol was approved by the institutional review board of the Seoul National University Hospital, Seoul, Korea, and all procedures were per-

Table I. Study schedule and treatment regimens for this open-label, 1-sequence, 3-period, 3-treatment crossover study conducted in healthy Korean male volunteers.*

Day 1	Days 6–7	Day 8	Days 12–21	Day 22
Mirodenafil 100 mg	Ketoconazole 400 mg once daily	Ketoconazole 400 mg + mirodenafil 100 mg	Rifampicin 600 mg once daily	Mirodenafil 100 mg

*Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days (period 3).

formed in accordance with the Good Clinical Practice Guideline[10] and the recommendations of the Declaration of Helsinki on biomedical research involving human subjects.[11] All subjects provided written informed consent before being enrolled in this study. The study was conducted at the Clinical Trials Center, Seoul National University Hospital. The laboratory tests were managed at the Department of Laboratory Medicine, Seoul National University Hospital. The plasma concentrations for mirodenafil and *N*-dehydroxyethyl mirodenafil were determined at the Department of Pharmacology and Clinical Pharmacology, Seoul National University Hospital, and Bio-Core Co., Ltd., Seoul, Korea, respectively.

Assay of Mirodenafil and *N*-Dehydroxyethylated Metabolite Levels in Plasma

Determination of Plasma Mirodenafil Concentration

The plasma mirodenafil concentration was determined using LC-MS/MS.[12,13] One hundred fifty microliters of plasma and 50 μL of internal standard (IS) solution (SK3304, 250 ng/mL in methanol [SK Chemicals Co., Ltd., Suwon, Korea]) were mixed thoroughly, 0.5 mL of acetonitrile containing 0.1% formic acid was added, and the mixture was centrifuged at 2000*g* for 10 minutes. The organic solvent was evaporated using a centrifugal evaporator (SpeedVac, Servant Instruments, Inc., Farmingdale, New York), and the remaining solids were reconstituted with 500 μL reconstitution solution (1 mM ammonium acetate, acetonitrile containing 0.1% formic acid, 50:50 vol/vol, pH 6.0) and then injected into the LC-MS/MS system for analysis. The plasma concentration of mirodenafil was determined from the standard curve by using the ratio between the peak area of mirodenafil and that of the IS.

The lower limit of quantitation (LLOQ) was 1 ng/mL, with a calibration curve ranging from 1 to 1000 ng/mL. The intra- and interday accuracy ranged from 105.4% to 112.7%, and the intra- and interday precision, expressed as %CV, was <5%, validating the plasma concentration analysis method over the given quantitation range.

Determination of Plasma N-Dehydroxyethylated Mirodenafil Concentration

The plasma *N*-dehydroxyethylated mirodenafil concentration was also determined by LC-MS/MS.[12,13] Two hundred microliters of plasma and 20 μL of IS solution (SK3304, SK Chemicals, 1 μg/mL in methanol) were mixed thoroughly, 0.5 mL of acetonitrile was added, and the mixture was centrifuged at 6625*g* for 5 minutes. The supernatant was diluted with 300 μL of mobile phase (1 mM ammonium acetate, acetonitrile, 30:70 vol/vol, pH 6.0) and then injected into the LC-MS/MS system for analysis. The plasma concentration of *N*-dehydroxyethylated mirodenafil was determined from the standard curve by using the ratio between the peak area of *N*-dehydroxyethylated mirodenafil and that of the IS.

The LLOQ was 1 ng/mL, with a calibration curve ranging from 1 to 1000 ng/mL. The intra- and interday accuracy ranged from 88.6% to 102.8%, and the intra- and interday precision, expressed as %CV, was <12%, validating the plasma concentration analysis method over the given range.

Pharmacokinetic Analysis

After discarding 1 mL of blood to remove the saline locked in an intravenous cannula inserted into a forearm vein of each subject, blood samples (8 mL) were drawn from the cannula before (0 hour) and at

1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 hours after mirodenafil administration and placed into sodium heparin-coated tubes. Then, 1 mL of saline was injected to prevent coagulation. Plasma was obtained by centrifugation at 2000*g* for 10 minutes at 4°C and was stored in polypropylene tubes at –20°C until measurement of plasma concentrations was conducted.

Pharmacokinetic analysis was performed by noncompartmental evaluation using WinNonlin Professional Network version 5.2 (Pharsight Corporation, Mountain View, California). The parameters C_{max} and T_{max} were directly obtained from the observed values. The terminal elimination rate constant (λ_z) was estimated by linear regression of the log-linear decline of the individual plasma concentration–time data. The $t_{1/2}$ was calculated as natural logarithm (ln)(2)/λ_z.

The individual AUC_{0-last} was calculated using the trapezoidal rule. $AUC_{0-\infty}$ was calculated as C_{last}/λ_z + AUC_{0-last}, where C_{last} is the last measurable concentration. The metabolic ratio was calculated by dividing the $AUC_{0-\infty}$ of *N*-dehydroxyethyl mirodenafil by the $AUC_{0-\infty}$ of mirodenafil.

This study was designed as a crossover study. Therefore, during analyses, body weight correction did not alter the geometric mean ratios, although the CIs may have been altered. Values for C_{max} and $AUC_{0-\infty}$ were calculated after correcting for milligram dose, and the same values for geometric mean ratios were obtained.

Tolerability

AEs were identified by asking general health-related questions of the subjects, such as, "Did you experience anything untoward?" or "Do you have any discomfort?" and by subject self-report throughout the study period. Physical examinations, which included the lungs, heart, and abdomen, were conducted at screening, predose, and 24 hours after drug administration on the pharmacokinetic-assessment days of every period: days 1, 8, and 22, and day of poststudy visit. ECGs, including ventricular rate, QRS, and PR and QTc intervals were evaluated at screening, predose, and at 2, 4, and 24 hours after drug administration on days 1, 8, and 22. In the second study period, additional ECGs were performed at 1, 3, 5, 8, and 12 hours after drug administration on day 8 and day of poststudy visit. Hematology, urinalysis, and clinical biochemistry, including liver function tests such as aspartate aminotransferase, alanine aminotransferase, and bilirubin, were performed at screening, predose, and 24 hours after dosing on days 1, 8, and 22, and day of poststudy visit. Vital signs, including heart rate and blood pressure, were also determined at regular intervals throughout the study: at screening, predose, and at 1, 2, 3, 4, 6, 8, 12, and 24 hours after drug administration on days 1, 8, and 22, and day of poststudy visit. Body temperatures were checked at screening and at poststudy visit. Hematology was analyzed by a Sysmex SE-9000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan), and urinalysis included dipstick conducted by US-3100R (Eiken Chemical Co., Ltd., Tokyo, Japan). Chemistry was detected by a Toshiba 200FR Neo Chemistry autoanalyzer (Toshiba Medical Systems Co., Ltd., Tokyo, Japan). These laboratory tests were performed after daily quality control tests of the equipment were conducted according to vendor specifications. All analyses were performed in the Department of Laboratory Medicine at Seoul National University Hospital, which is certified by the College of American Pathologists.

Statistical Analysis

Statistical analysis was performed using SPSS 12.0 (SPSS Korea, Seoul, Korea). Pharmacokinetic parameters of mirodenafil in the CYP3A4-inhibited or -induced state resulting from ketoconazole or rifampicin pretreatment were compared. A mixed-model ANOVA was employed with fixed effects for the period and treatment, and with random effects for subjects nested within a sequence. For the comparison of pharmacokinetic characteristics between mirodenafil alone and mirodenafil coadministered with ketoconazole or rifampicin, C_{max}, $AUC_{0-\infty}$, and $t_{1/2}$ were log-transformed and tested by mixed-model ANOVA. The mean differences and 90% CIs were back-transformed to obtain the geometric mean ratios and CIs for those ratios.[14]

RESULTS

Subjects

The mean (SD) age of the subjects was 23.2 (2.76) years (range, 19–29 years). The subjects' mean weight was 69.3 (6.50) kg (range, 61.0–84.0 kg), and body mass index was 22.4 (1.77) kg/m^2 (range, 20.0–26.0 kg/m^2). Nineteen subjects were enrolled, but 1 subject dropped out due to consent withdrawal; he did not visit the Clinical Trials Center for a scheduled appointment due to personal reasons, and he did not complete the third treatment period. This subject was

removed from the study because of these protocol violations and was replaced. Pharmacokinetics were evaluated for the 18 subjects who completed the study. Tolerability profiles were assessed for the 19 subjects who were administered study drugs.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed in the 18 subjects who completed the study. The mean plasma concentration–time profiles of mirodenafil after all 3 study periods are shown in **Figure 1**. The pharmacokinetic parameters of mirodenafil after ketoconazole and rifampicin pretreatment are compared with those of mirodenafil administration alone in **Table II**.

Mirodenafil Alone

Mirodenafil administered alone had a median (range) T_{max} of 1.26 hours (1.00–2.50 hours) after a dose of mirodenafil 100 mg, and a mean (SD) $t_{1/2}$ of 1.96 (0.55) hours. The mean C_{max} was 373.4 (171.3) ng/mL and the mean $AUC_{0-\infty}$ was 931.4 (404.6) ng · h/mL when mirodenafil was administered alone. The $AUC_{0-\infty}$ of *N*-dehydroxyethyl mirodenafil was 492.4 (224.9) ng · h/mL.

Mirodenafil After Pretreatment With Ketoconazole

The T_{max} was delayed to 2.00 hours (range, 1.00–3.00 hours) when mirodenafil was administered after pretreatment with ketoconazole for 3 days. The mean (SD) $t_{1/2}$ was 3.35 (0.49) hours. The mean C_{max} was 991.6 (281.2) ng/mL and the mean $AUC_{0-\infty}$ was 4557.1 (1606.3) ng · h/mL (**Table II, Figure 2**).

The mirodenafil concentration was higher in the presence of ketoconazole: 2.83-fold (90% CI, 2.12–3.77) for C_{max} and 5.04-fold (90% CI, 3.78–6.72) for $AUC_{0-\infty}$ (**Table II**). The mean (SD) $AUC_{0-\infty}$ of *N*-dehydroxyethyl mirodenafil was 496.7 (305.2) ng · h/mL. The metabolic ratio of mirodenafil to

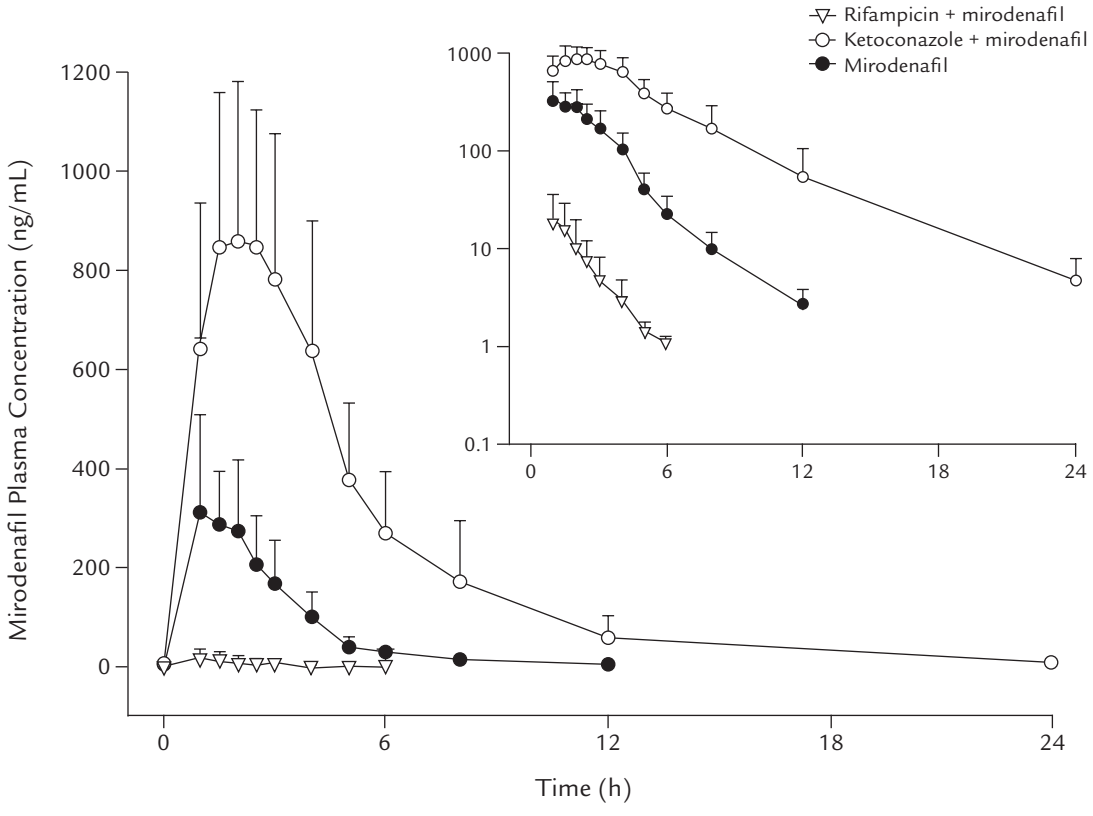

Figure 1. Mean (SD) plasma concentration–time profiles of mirodenafil after oral administration of mirodenafil, ketoconazole with mirodenafil, and rifampicin with mirodenafil.

Table II. Comparison of pharmacokinetic parameters after administration of a single 100-mg dose of mirodenafil alone or in combination with ketoconazole or rifampicin pretreatment (n = 18).* Data are mean (SD), unless otherwise specified.

Parameter	Mirodenafil Alone	Ketoconazole + Mirodenafil	Rifampicin + Mirodenafil	Ratio of Geometric Means (90% CI)	
				Ketoconazole + Mirodenafil/ Mirodenafil	Rifampicin + Mirodenafil/ Mirodenafil
C_{max}, ng/mL	373.4 (171.3)	991.6 (281.2)†	20.2 (17.4)†	2.83 (2.12–3.77)‡	0.05 (0.03–0.07)‡
$AUC_{0-\infty}$, ng · h/mL	931.4 (404.6)	4557.1 (1606.3)†	38.3 (28.5)†	5.04 (3.78–6.72)‡	0.03 (0.02–0.05)‡
T_{max}, median (range), h	1.26 (1.00–2.50)	2.00 (1.00–3.00)†	1.00 (1.00–2.00)	–	–
$t_{1/2}$, h	1.96 (0.55)	3.35 (0.49)†	1.01 (0.26)†	–	–

*Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days (period 3).
† $P < 0.05$, comparison of values using ANOVA.
‡ $P < 0.05$, comparison of values using the paired *t* test.

N-dehydroxyethyl mirodenafil with ketoconazole pretreatment was 0.12 (0.10) compared with 0.54 (0.14) for mirodenafil alone (**Table III, Figure 2**).

Mirodenafil After Pretreatment With Rifampicin

The T_{max} was 1.00 hour (range, 1.00–2.00 hours) when mirodenafil was given after pretreatment with rifampicin for 10 days. The mean (SD) $t_{1/2}$ was 1.01 (0.26) hours. The mean C_{max} was 20.2 (17.4) ng/mL and the mean $AUC_{0-\infty}$ was 38.3 (28.5) ng · h/mL (**Table II, Figure 2**).

The mirodenafil concentration was lower in the presence of rifampicin: 0.05-fold (90% CI, 0.03–0.07) for C_{max} and 0.03-fold (90% CI, 0.02–0.05) for $AUC_{0-\infty}$ (**Table II**). The mean (SD) $AUC_{0-\infty}$ of *N*-dehydroxyethyl mirodenafil was 49.3 (29.6) ng · h/mL. The metabolic ratio of mirodenafil to *N*-dehydroxyethyl mirodenafil with rifampicin pretreatment was 1.51 (0.61) compared with 0.54 (0.14) for mirodenafil alone (**Table III, Figure 2**).

Tolerability

Fourteen of 19 subjects reported >1 AE. In total, 40 AEs were reported. Among these, 12 cases of headache, 6 of nasal congestion, 2 of feeling hot, 2 of epistaxis, and 1 each of dizziness, nausea, and somnolence were considered by the investigators to be related to mirodenafil administration (**Table IV**).

Most of the AEs related to the study drug were mild, but 2 cases of headache and 1 case each of influenza-like illness, nausea, and somnolence were of moderate intensity. These were considered to be related to mirodenafil administration, except for the influenza-like illness.

The AEs judged to be unrelated to the administration of mirodenafil, ketoconazole, or rifampicin after considering the onset of the AE, the time interval of drug administration, and the time course of the AE included: headache (3 cases), nasal congestion (3), epistaxis (2), conjunctival hyperemia (1), cough (1), dizziness (1), postural dizziness (1), epigastric discomfort (1), influenza-like illness (1), nausea (1), and rhinorrhea (1). In the mirodenafil with ketoconazole pretreatment period (period 2), there was a greater number of AEs compared with the other treatment periods (**Table IV**). This finding was most notable in regard to the number of headaches: 9 cases of headache were reported in the second study period, 4 cases

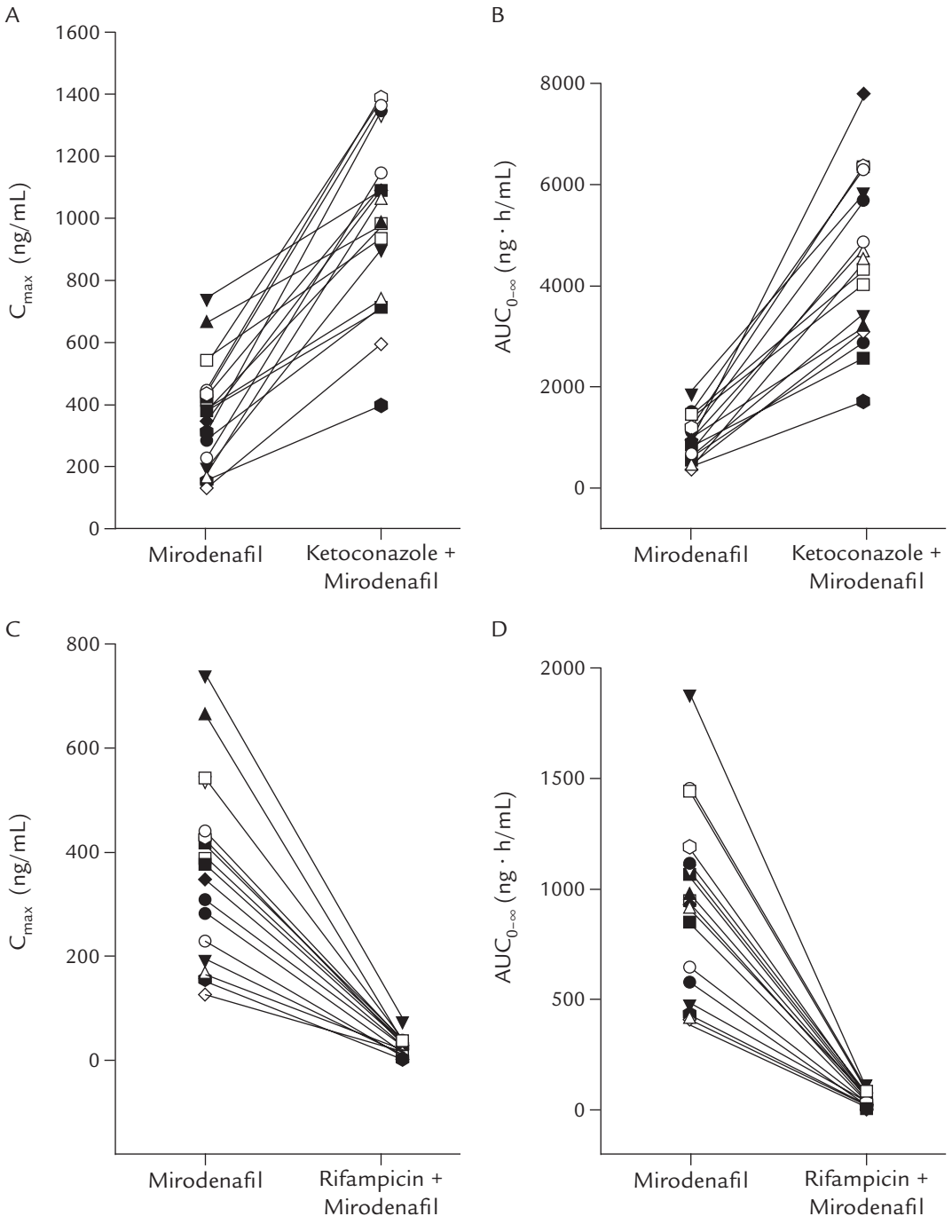

Figure 2. (A) C_{max} and (B) $AUC_{0-\infty}$ of mirodenafil administered alone and with ketoconazole pretreatment. (C) C_{max} and (D) individual $AUC_{0-\infty}$ of mirodenafil administered alone and with rifampicin pretreatment.

Table III. Ratio of the AUCs of *N*-dehydroxyethyl mirodenafil and mirodenafil after administration of a single 100-mg dose of mirodenafil alone or in combination with steady-state ketoconazole or rifampicin.* Values are mean (SD).

Parameter	Mirodenafil	Ketoconazole + Mirodenafil	Rifampicin + Mirodenafil
N-dehydroxyethyl mirodenafil/mirodenafil	0.54 (0.14)	0.12 (0.10)† 0.21-fold‡	1.51 (0.61)† 2.9-fold‡
P		0.024	<0.01

*Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days (period 3).
† $P < 0.05$, comparison of values using ANOVA.
‡ Fold change of *N*-dehydroxyethylated mirodenafil/mirodenafil compared with mirodenafil alone.

of headache were reported in the first study period, and 2 cases of headache were reported in the third study period (**Table IV**). The discontinued subject reported cough, headache, and influenza-like illness. These AEs were judged to be unrelated to the administration of the study drugs and resolved without medical intervention.

There were no clinically significant findings from physical examinations, 12-lead ECGs, vital signs, or laboratory tests.

DISCUSSION

After pretreatment with ketoconazole, the $AUC_{0-\infty}$ of mirodenafil was increased 5.04-fold and the metabolic AUC ratio was decreased 0.21-fold compared with mirodenafil administered alone. After pretreatment with rifampicin, the $AUC_{0-\infty}$ of mirodenafil was decreased 0.03-fold and the metabolic AUC ratio was increased 2.9-fold. This result provides evidence of CYP3A4-mediated drug–drug interactions with mirodenafil.

The effects of ketoconazole and rifampicin on the pharmacokinetics of mirodenafil seen in this study are comparable to those reported for several CYP3A4 substrates on other PDE5 inhibitors.[15,16] PDE5 inhibitors such as sildenafil, vardenafil, tadalafil, and udenafil are also reportedly metabolized by CYP3A4 to *N*-dealkylated metabolites.[15–18] Several studies have reported that the drug–drug interactions of these PDE5 inhibitors are mediated by CYP3A4.[16,19–22] Sildenafil 100 mg exhibited an 11-fold (90% CI, 9.0–12.0) increase in AUC and a 3.7-fold (90% CI, 3.2–4.9) increase in C_{max} when administered with ritonavir 500 mg twice daily, which is metabolized by CYP3A4.[16] Vardenafil 5 mg coadministered with ketoconazole 200 mg once daily in healthy volunteers resulted in a 4-fold increase in the C_{max} and a 10-fold increase in the AUC of vardenafil.[19] Ketoconazole 400 mg once daily increased the AUC of a single 20-mg dose of tadalafil by 4.1-fold compared with the administration of tadalafil alone. In the same study, ketoconazole 200 mg once daily increased the C_{max} and AUC of a single 10-mg dose of tadalafil by 1.2- and 2.0-fold, respectively.[18] Rifampicin reduced the AUC of a 10-mg dose of tadalafil 0.11-fold, relative to the value for tadalafil 10 mg alone.[19,23]

It has been postulated that CYP3A4 and P-glycoprotein (P-gp) have some degree of substrate overlap and co-localize in the small intestine.[6,24–26] P-gp is an adenosine-5′-triphosphate-dependent efflux transporter and an important molecular determinant of oral bioavailability.[6,26] Ketoconazole and rifampicin are, respectively, an inhibitor and an inducer of P-gp.[6,27–31] Ketoconazole inhibited P-gp at 6 μM for a 50% inhibitory concentration in an in vitro study.[27] Rifampicin increased the contents of intestinal P-gp by 3.5-fold in healthy volunteers.[29] Thus, it is possible that if mirodenafil is also a substrate of P-gp, the drug–drug interactions of mirodenafil with ketoconazole and rifampicin would not only be due to an interaction with CYP3A4, but also with P-gp (No previous research addressing this question was identified in a search of the published literature in PubMed using the terms *mirodenafil*, *ketoconazole*, *rifampicin*, *drug interaction*, and *P-gp*). Udenafil and vardenafil are

Table IV. Adverse events (AEs) per system organ class and study treatment administered to healthy Korean male volunteers (N = 19).*

	Mirodenafil Alone		Ketoconazole + Mirodenafil		Rifampicin + Mirodenafil†	
System Organ Class/AE	No. of Events	No. (%) of Subjects	No. of Events	No. (%) of Subjects	No. of Events	No. (%) of Subjects
General disorders and administration site conditions						
Feeling hot	–	–	1	1 (5.26)	1	1 (5.56)
Influenza-like illness	–	–	1	1 (5.26)	–	–
Gastrointestinal disorders						
Nausea	–	–	2	2 (10.5)	–	–
Epigastric discomfort	1	1 (5.26)	–	–	–	–
Eye disorders						
Conjunctival hyperemia	–	–	1	1 (5.26)	–	–
Nervous system disorders						
Headache	4	3 (15.8)	9	9 (47.4)	2	2 (11.1)
Dizziness	–	–	1	1 (5.26)	–	–
Postural dizziness	–	–	1	1 (5.26)	–	–
Somnolence	–	–	1	1 (5.26)	–	–
Respiratory, thoracic, and mediastinal disorders						
Nasal congestion	2	2 (10.5)	5	4 (21.1)	2	2 (11.1)
Cough	–	–	1	1 (5.26)	–	–
Rhinorrhea	–	–	1	1 (5.26)	–	–
Vascular disorders						
Epistaxis	–	–	4	3 (15.8)	–	–
Total	7	6 (31.6)	28	13 (68.4)	5	3 (16.7)

*Each subject received 100 mg of mirodenafil in each of 3 study periods: mirodenafil alone (period 1); mirodenafil after pretreatment with ketoconazole 400 mg once daily for 3 days (period 2); and mirodenafil after pretreatment with rifampicin 600 mg once daily for 10 days (period 3).

† n = 18 due to 1 subject discontinuing the study.

both known CYP3A4 substrates[18,19] and they were also found to be P-gp substrates in 2 in vitro studies.[32,33] However, not all CYP3A4 substrates are P-gp substrates.[34] There is a need for further study to determine the extent of the effect of P-gp on the pharmacokinetics of mirodenafil.

The most commonly reported AEs (>10% of subjects) were headache (period 1, 15.8%; period 2, 47.4%; period 3, 11.1%), nasal congestion (period 1, 10.5%; period 2, 21.1%; period 3, 11.1%), epistaxis (period 2, 15.8%), and nausea (period 2, 10.5%). These are commonly reported AEs of PDE5 inhibitors in clinical trials and in clinical applications.[1,2,35] Twenty-eight AEs were reported in period 2 (in 68.4% of subjects), during which systemic exposure to mirodenafil was highest, whereas 7 AEs were reported in period 1 (in 31.6% of subjects) and 5 AEs were reported in period 3 (in 15.7% of subjects) (**Table IV**). This finding suggests an association between mirodenafil concentration and AEs. Despite the moderately higher systemic exposure of mirodenafil with ketoconazole pretreatment, no serious AEs nor any AEs that required the discontinuation of the study were reported.

There were 2 considerations in determining the drug administration time in the study design related to the pharmacologic time course of inhibition and induction. First, in the case of ketoconazole, the time course of CYP3A4 inhibition was considered. The inhibitory effect of ketoconazole on intestinal CYP3A4 extended beyond the residence time of the drug in the small intestine.[36] The small intestine transit time for the inhibition of CYP3A4 by ketoconazole after oral administration was 3.5 hours,[36,37] and the T_{max} of mirodenafil after oral administration was 2.5 hours.[2] Therefore, it was determined that a 1-hour interval between ketoconazole and mirodenafil administration would result in the maximum inhibitory effect of ketoconazole. Second, the time course of the induction of CYP3A4 was taken into account. It has been reported that full induction is reached 1 week after starting rifampicin,[38] so the drug–drug interaction was evaluated after 10 days of rifampicin administration to ensure full inhibition.

This study has several limitations. First, it was conducted in a small number of healthy male volunteers; therefore, the results might not directly apply to a larger population or to special population groups. Second, the effect of CYP3A genetic polymorphisms in the subjects was not considered in this study. Genetic polymorphism of CYP3A may contribute to the disposition of PDE5 inhibitors, especially vardenafil.[39] Further research is needed to investigate the contribution of such CYP3A genetic polymorphisms to drug–drug interactions with mirodenafil.

CONCLUSIONS

Both ketoconazole and rifampicin were found to have a significant effect on the pharmacokinetics of mirodenafil. Ketoconazole increased the systemic exposure of mirodenafil, whereas rifampicin reduced it. The dose of mirodenafil may require adjustment when coadministered with drugs that alter CYP3A4 activity.

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