Department of Surgery[1], Department of Pharmacology & Institute of Biomedical Sciences[2], Department of Internal Medicine[3], College of Medicine, Hanyang University, Seoul, South Korea; Department of Pathology and Laboratory Medicine[4], College of Medicine, University of Pennsylvania, Philadelphia, USA

A sensitive validated LC-MS/MS method for quantification of itraconazole in human plasma for pharmacokinetic and bioequivalence study in 24 Korean volunteers

Si-Youn Rhim[1], Jin-Hee Park[2], Yoo-Sin Park[2], Dong-Sun Kim[3], Min-Ho Lee[3], Leslie M. Shaw[4], Ju-Seop Kang[2]

Received October 24, 2007, accepted July 21, 2008

Ju-Seop Kang, Department of Pharmacology & Institute of Biomedical Sciences, College of Medicine; Department of Bioengineering, Hanyang University, Seoul 133–791, Korea
jskang@hanyang.ac.kr

Pharmazie 64: 71–75 (2009) *doi: 10.1691/ph.2009.7795*

A rapid and highly sensitive liquid chromatography/ electrospray ionization tandem mass spectrometric method (LC/ESI-MS/MS) for itraconazole determination in human plasma was validated and applied to pharmacokinetic and bioequivalence study in humans. In a randomized crossover design with a 1-week period, each subject received a 200 mg itraconazole capsule. The analytical procedures involved a less time-consuming, simple protein precipitation with methyl t-butyl ether and separation by HPLC. The ionization was optimized using electrospray ionization (ESI) with positive ion mode and selectivity was achieved by MS/MS analysis, m/z 705.3 → 392.4 and m/z 374.3 → 141.0 for itraconazole and internal standard (IS), respectively. The standard calibration curves showed good linearity within the range of 1 (LLOQ) to 500 ng/mL for itraconazole in human plasma with a correlation coefficient $r \geq 0.9952$. The retention times of itraconazole (0.9 min) and IS (0.84 min) suggest the high throughput of the proposed method. No significant metabolic compounds were found to interfere with the analysis. The coefficient of variation values of both intra- and inter-day were below 13.7% and 10.9%, respectively. Intra- and inter-day accuracies were 95.6–108.2% and 86.6–117.5%, respectively. This method was successfully applied for pharmacokinetic and bioequivalence study in 24 healthy human subjects by analysis of blood samples taken up to 72 h after an oral dose of 200 mg of itraconazole.

1. Introduction

Understanding of the pharmacokinetic properties of systemic antifungals is critical to their appropriate application (Lyman et al. 1992; Kauffman 1996). The pharmacokinetics of orally administered itraconazole (ITZ) in humans is characterized by dose dependent (Hardin et al. 1988), wide interindividual variations in absorption and plasma concentration (Hardin et al. 1988; Suarez-Kurtz et al. 1999; Conway et al. 2004; Lohitnavy et al. 2005), and significant difference in pharmacokinetic parameters in different ethnic population when ITZ was administered together with food (Yeates et al. 1995; Yun et al. 2006).

For quantification of ITZ in human blood, several HPLC methods have been developed following Warnock et al. (1988). Most HPLC methods, however, have used complicated, time-consuming extraction procedures and unspecific detection techniques such as fluorescence detection or ultraviolet detection (UVD). Using these conventional detection techniques, a long retention time was needed to avoid analytical interference from metabolites of ITZ. In most HPLC methods the LLOQ was higher than 10 ng/ml (Warnock et al. 1988; Gubbins et al. 1998; Al-Rawithi et al. 2001; Koks et al. 2002). More recently developed HPLC methods using column-switching technique (Uno et al. 2006) or fluorescence detection (Wong et al. 2003; Srivatsan et al. 2004) presented a relatively low LLOQ of 3–5 ng/ml, but again, had the disadvantage of long run time. Because the need to minimize the analytical time and to maximize the specificity and sensitivity is a key driver in bioanalysis (Ackermann et al. 2002), LC-MS/MS has become the preferred choice for determination of small molecule drugs (Jemal 2000; Xu et al. 2007). However, only a few researchers reported on the use of LC-MS (Carrier et al, 2000; Yao et al., 2001) or LC-MS/MS methods (Vogeser et al. 2003; Kousoulos et al. 2006) for the determination of ITZ.

In this paper, we present a faster method with lower detection limit for the determination of ITZ in human plasma. The proposed LC-MS/MS method allowed very sensitive and reliable determination of ITZ in a pharmacokinetic and bioequivalence study after oral administration of the two 100 mg ITZ capsules in 24 healthy male volunteers.

2. Investigations and results

2.1. Separation

Simple preparation procedures including protein precipitation, evaporation of extracted sample and reconstitution with mobile phase were used before reverse phase HPLC separation. The retention times of ITZ and clebopride (IS) were 0.9 and 0.84 min, respectively. Blank human plasma had no significant endogenous peaks at the retention time of ITZ or IS in the mass chromatogram (Fig. 1A). Blank plasma spiked with 100 ng/mL of IS (Fig. 1B), blank plasma spiked with 1.0 mg/mL of calibration standard of ITZ and 100 ng/mL of IS (Fig. 1C), and subject's plasma obtained 3 h after a single oral administration of 200 mg ITZ spiked with IS (Fig. 1D) showed excellent peak shape for ITZ and IS.

To avoid interference from exogenous/endogenous compounds co-eluted with the target compound, MS/MS detection (termed tandem MS detection) which consists of a three quadrupole system, offering unique selectivity against matrix background and requires very limited sample preparation was performed. Ionization of analytes was carried out using the electrospray ionization (ESI) technique with positive polarity and multiple reaction monitoring (MRM) mode. Full-scan precursor ion mass spectra showed that the predominant ions of ITZ and IS were the protonated molecules, $[M+H]^+$, of m/z 705.3 and 374.2, respectively (Fig. 2A). After collision-induced dissociation, the most abundant ion in the product ion mass spectrum was at m/z 705.3 → m/z 392.4 for ITZ and m/z 374.2 → m/z 141.0 for IS (Fig. 2B). The most suitable collision energy was determined by observing the response obtained vs selectivity response for the fragment ion for each compound. The best collision energies set were 49.0 eV for ITZ and 16.0 eV for IS obtaining fragments m/z 392.4 and m/z 141.0 from the respective protonated compound.

2.2. Method validation and linearity of calibration

The standard calibration curves showed good linearity within the range of 1 (LLOQ) to 500 ng/mL using least-squares regression analysis. The correlation coefficients for calibration plots were equal to or better than 0.9952. The relationship between the concentration of standard and peak area ratio of ITZ and IS was also shown to be linear from 1 to 500 ng/mL. Intra- and inter-day precisions were determined for calibration curves prepared on the same day ($n = 5$) and different days ($n = 5$). As shown in Table 1, this method allowed good precision and accuracy. The coefficient of variation values of both intra- and inter-day were below 13.7% and 10.9%, respectively. Intra- and inter-day accuracies were 95.6–108.2% and 86.6–117.5%, respectively. Under the analytical condi-

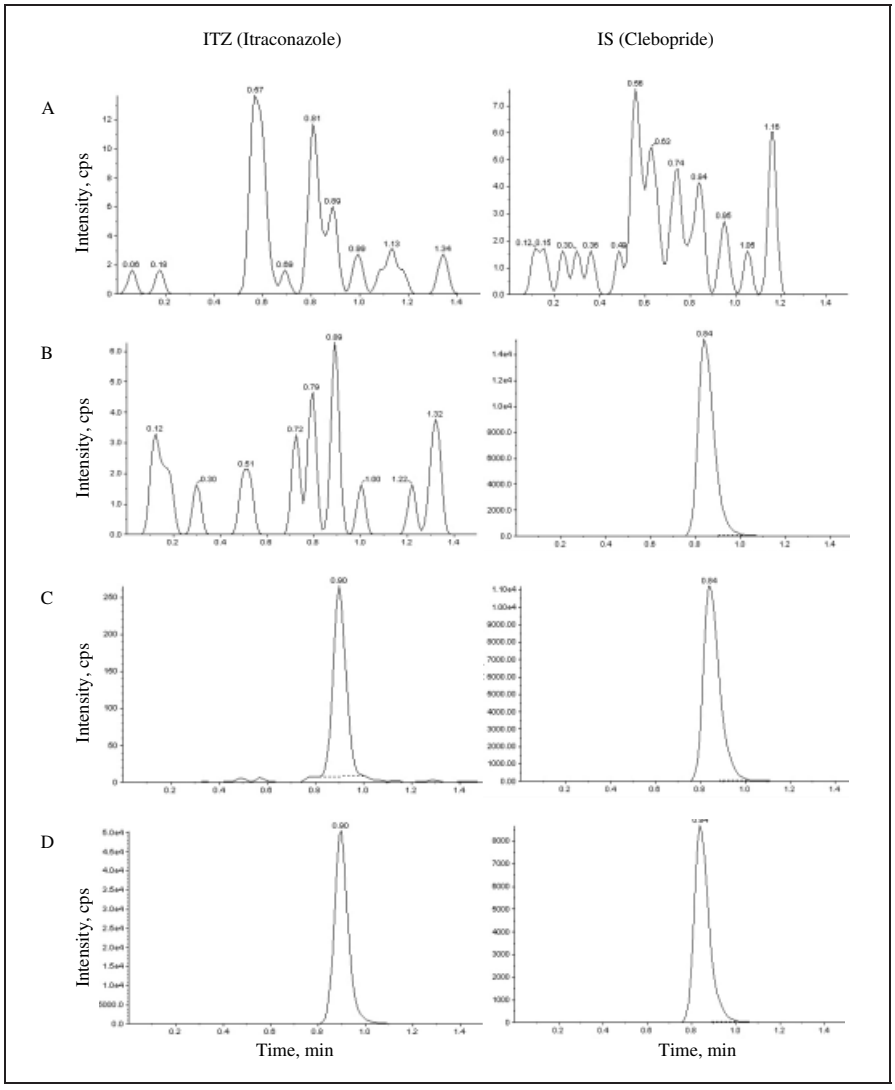

Fig. 1:
Chromatograms of (A) blank plasma, (B) with IS (100 ng/mL), (C) with IZT (LLOQ, 1 ng/ml) and IS, and (D) human plasma taken 3 h after a single oral administration of 200 mg ITZ spiked with IS

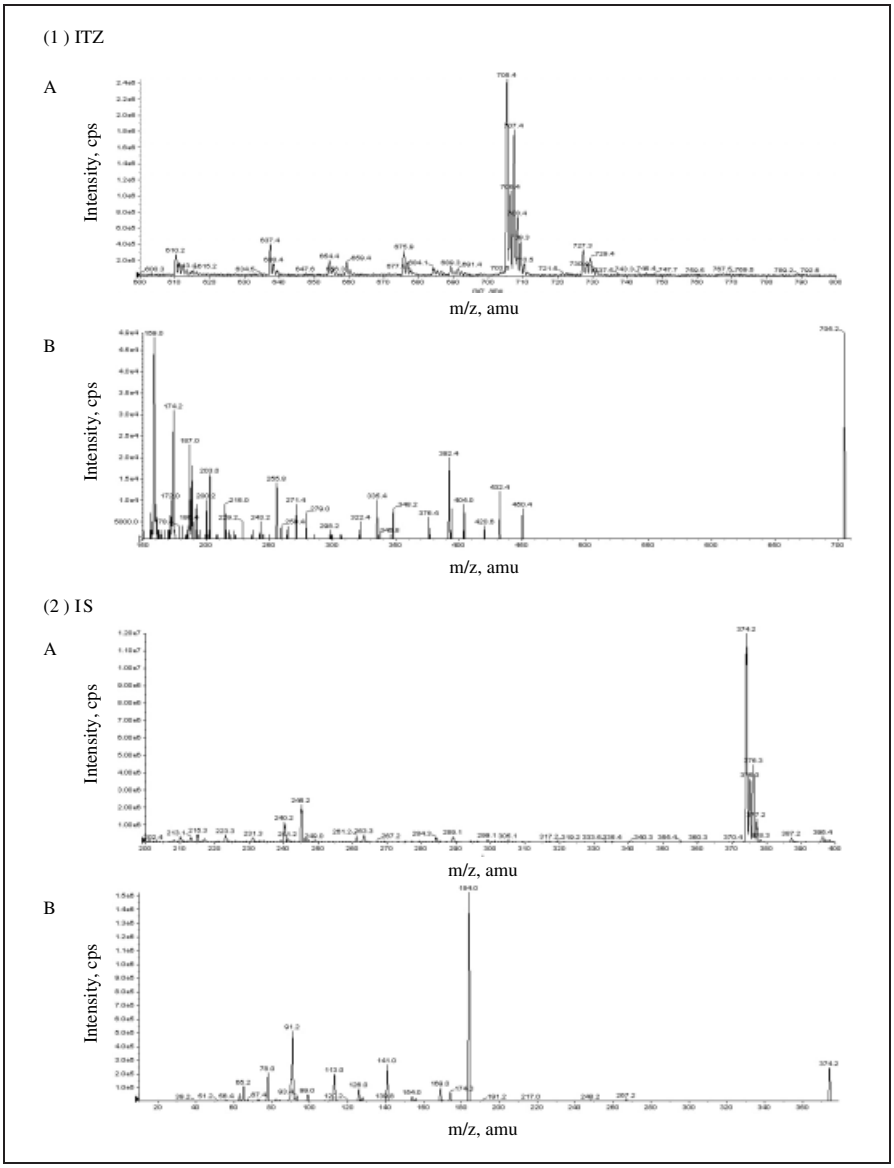

Fig. 2:
Full-scan mass spectra of (A) precursor ions and (B)product ions of (1) itraconazole(ITZ) and (2) cleboptride(IS)

Table 1: Precision and accuracy for analysis of itraconazole in human plasma, n = 5

Nominal Conc. (ng/mL)	Precision (CV%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
1(LLOQ)	13.69	10.91	107.70	117.52
3	3.89	9.77	108.24	93.23
50	2.01	4.51	101.10	95.55
400	1.66	3.70	95.57	86.61

LLOQ = Lower limit of quantification; CV = coefficient of variation.

tions described, the lowest limit of quantification (LLOQ), defined as the lowest concentration of ITZ at which both precision and accuracy were less than or equal to 20% (Guidance for industry, 2001), was 1 ng/mL.

2.3. Clinical application in healthy subjects

The proposed method was applied to the determination of ITZ in plasma samples for the purpose of establishing the pharmacokinetic and bioequivalence study of 200 mg ITZ formulations in 24 healthy Korean volunteers. In early pharmacokinetic studies, a dose of ITZ was normally administered immediately after a standardized breakfast to increase its bioavailability (Hardin et al. 1988; Barone et al. 1998). However, recently, it was figured out that consumption of a bread meal before administering itraconazole caused a significant increase in the bioavailability and maximum plasma concentration. Conversely, the consumption of a rice meal before administering ITZ caused a significant decrease in ITZ bioavailability (Yun et al. 2006). In this study on rice meal eating Korean subjects, therefore, ITZ was administered to the subjects in the fasted state.

The pharmacokinetic parameters for the reference (Sporanox™ capsule, Janseen Korea Co., South Korea) and test drug (CTItrazole™ tablets, CTBIO Pharm Co., South Korea) obtained are described as follows. The profiles of the plasma ITZ concentration vs time is shown in Fig 3. Plasma concentrations of ITZ were in the standard curve range and remained above the LLOQ (1 ng/mL) for the entire sampling period except 2 subjects at 1 h and 1 subject at 72 h after dosing. Even though the bioavailability of orally administered ITZ is characterized by considerable interindividual variation, the plasma profiles of the mean ITZ concentration vs time after oral administration of a single dose of both formulations in 24 subjects exhibited closely similar patterns. The mean estimated pharma-

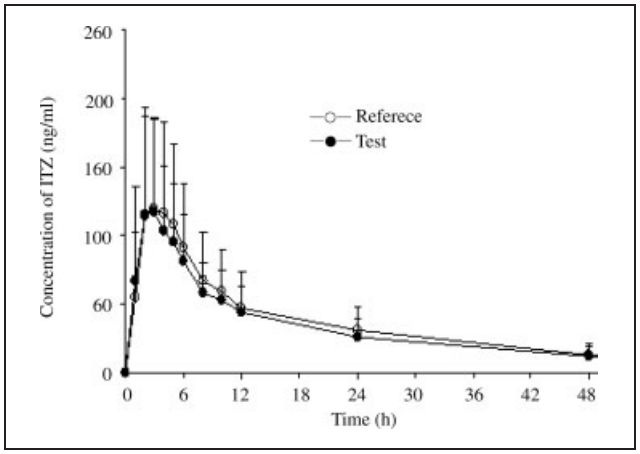

Fig. 3: Profiles of mean (± S.D., n = 24) plasma concentrations versus time of two itraconazole(ITZ; reference vs test) formulations in healthy 24 volunteers after oral administration of single 200 mg ITZ formulations

cokinetic parameters derived from the plasma concentration profiles of ITZ are shown in Table 2. The maximum plasma concentration (C_{max}) that is independent to sampling time was 153.4 (±74.0) ng/mL for the reference and 144.4 (±64.8) ng/mL for the test. Median time (range) to observed maximum plasma concentration (T_{max}, h) was 3.0 (2.0–6.0) h for both of the reference and test, and elimination half-life ($T_{1/2}$, h) was 19.9 (±4.8) h for the reference and 20.2 (±5.5) h for the test. In previous reports, absorption of ITZ after oral administration is rapid with 156–319 ng/mL of C_{max} and 2.0–4.7 hours of T_{max} in fasting conditions. Under noninduced conditions, ITZ elimination half-life($T_{1/2}$) averaged between 18–22.5 h (Barone et al. 1998; Hardin et al. 1988; Suarez-Kurtz et al. 1999). Our experimental results agreed well with those studies.

The mean ratio of the AUC_t divided by AUC_∞ was above 90.5% and 91.0% of reference and test, respectively, ranging within the limits of the FDA (80–125%) (Chen et al. 2001; Hyder 2001; Shargel et al. 2005). The 90% confidence interval (CI) of test/reference percent ratios were 96.0% (89.35–103.15%) for C_{max}, 96.6% (84.82–107.85%) for $AUC_{72\,h}$ and 98.6%(85.34–108.27%) for AUC_∞. No differences between the reference and test drugs were detected (p-value greater than 0.05 for the formulation effect in all tested parameters). Therefore, the 2 types of 200 mg ITZ formulations were considered to be bioequivalent according to pharmacokinetic results obtained from concentration-time profiles of ITZ in 24 healthy volunteers.

Table 2: Pharmacokinetic parameters (mean ± S.D. of n = 24) of 2 types of 200 mg IZT formulations

Parameters	Reference	Test
$AUC_{72\,h}$ (μg · h/mL)	2.099 ± 1.118	1.915 ± 0.896
AUC_∞ (μg · h/mL)	2.336 ± 1.264	2.119 ± 1.029
Extrapolation (%)	9.5 ± 5.1	9.0 ± 5.4
C_{max} (μg /mL)	0.153 ± 0.074	0.144 ± 0.065
T_{max} (h)	3.2 ± 1.3(2.0–6.0)	3.3 ± 1.4 (2.0–6.0)
$T_{1/2}$ (h)	19.9 ± 4.8	20.2 ± 5.5
Ke (h^{-1})	0.0348 ± 0.0016	0.0343 ± 0.0012

AUC = area under plasma concentration-time curve; C_{max} = maximal plasma concentration; T_{max} = time for the maximal plasma concentration; $T_{1/2}$ = half-life; Ke = elimination rate constant

2.4. Conclusion

The proposed method combining a simplified plasma extraction procedure and highly sensitive HPLC/ESI-MS/MS provided a rapid and sensitive detection of ITZ in human blood. We achieved lower LLOQ (1 ng/ml) and shorter retention times (0.9 min for ITZ, 0.84 min for IS) than previous reports using LC/MS or LC-MS/MS (Carrier et al. 2000; Vogeser et al. 2003; Kousoulos et al. 2006). The precision and accuracy for calibration and QC samples were well within the acceptable limits. This method was sensitive enough for ITZ to monitor their plasma concentration up to 72h and afforded us a successful application in pharmacokinetic and bioequivalence study of the two types of 200 mg ITZ formulations in 24 healthy Korean volunteers.

3. Experimental

3.1. Chemicals and reagents

Itraconazole (ITZ, MW = 705.3 g/mol) and clebopride (IS, MW = 373.88 g/mol) were purchased from Neuland Laboratories Co. (Ameerpet, AP, India). Acetonitrile (ACN), HPLC grade methanol, methyl t-butyl ether were purchased from Merck Co. (Darmstadt, Germany). Analytical grade glacial acetic acid was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other reagent and solvents used were of analytical grade. All aqueous solutions and buffers were prepared using deionized and doubly distilled water using a Milli-Q plus system (Millipore Co. USA). Reference (Sporanox™ capsule, Janseen Korea Co. South Korea) and test drug (CTItrazole™ tablets, CTBIO Pharm Co. South Korea) containing 200 mg itraconazole per tablets and 100 mg per capsule were used.

3.2. Stock solutions and standards

Stock solutions and ITZ and IS were prepared with 50% tetrahydrofuran solution to a final concentration of 1.0 mg/mL and stored at −20 °C. A set of seven non-zero calibration standards, ranging from 1 ng/mL to 500 ng/mL, was prepared in blank human plasma. The quality control (QC) samples were prepared in blank human plasma at concentrations of 1 (LLOQ), 3 (low), 50 (medium) and 400 ng/mL (high). Blank human plasma was tested before spiking to ensure that no endogenous interference was found around retention times of ITZ and IS.

3.3. Preparation of plasma samples

0.02 mL of IS working solution (100 ng/mL of IS) and 5 mL of methyl t-butyl ether were added to the 1 mL aliquot of human plasma and vortexed for 10 min. After centrifugation at 4,000 rpm for 5 min, 0.5 mL of upper organic layer was evaporated to dryness under nitrogen gas at 40 °C, reconstituted in 0.15 mL of 50% methanol. The upper layer (5 μL) was directly injected into the LC-MS/MS system.

3.4. LC-MS/MS conditions and quantifications

The LC system used was a Nanospace series SI-2 (Shiseido Co., Ltd, Japan) chromatograph equipped with an Nanospace SI-2 Autosampler (Shiseido Co., Ltd, Japan) and Nanospace SI-2 degasser (Shiseido Co., Ltd, Japan) and Peak Simple LC Data System (Lab Alliance Co., State College, PA, USA) with Analyst 1.4 (Applied Biosystems). Mass spectrometric analysis was performed using an API 2000™ MS system (Applied Biosystems, Foster City, CA, USA) equipped with a turbo ion spray (ESI, 5000.0 V) interface operating in the positive ion mode with a cross-flow counter electrode. This system was set to the multiple reaction monitoring (MRM) mode, that is, selecting precursor ions, dissociating them and finally analyzing the product ions reaching great selectivity and sensitivity. The analytical column was a YMC hydrosphere C18 column (2.0 × 50 mm i.d., 3 μm; YMC Co., Ltd, Japan). The mobile phase consisted of acetonitrile and 1 mM ammonium acetate (90 : 10, v/v). The flow rate was 0.25 mL/min and the injection volume was 5 μL. The dwell time per transition was set at 0.2 s, and the source temperature was set at 350 °C with ultra-high-purity nitrogen as the curtain gas (45.0 L/min) and collision gas (5.0 L/min). The strongest fragment of each compound was selected and used as Q3 ion to be monitored. Unit resolution was used for both Q1 and Q3 mass detection. The ion source parameters were set as follows; curtain gas = 45 p.s.i., collision gas = 5 p.s.i., ion spray voltage = 5500 V, temperature = 350 °C, ion source (GS1) = 55 p.s.i. and ion source (GS2) = 55 p.s.i. No significant interferences at the retention times of itraconazole or IS were observed in the mass chromatograms of blank plasma.

3.5. Assay validation

Calibration curves were based on peak area ratios of ITZ to IS for seven calibration standards over the range of 1–500 ng/mL for ITZ in human plasma analyzed in duplicate. Linearity was determined to assess the performance of the method. Linear least-squares regression with a weighting index of $1/x^2$ was performed on the peak area ratios of ITZ to IS versus ITZ nominal concentrations of the seven plasma standards in duplicate to generate a calibration curve. Accuracy and precision were based on assay of five replicates of QC samples analyzed on five different days and calculated using one-way ANOVA.

3.6. Pharmacokinetic and bioequivalence studies in healthy volunteers

For comparing two formulations, we used the most common statistical design, that is, a randomized, two-period, two-sequence, and crossover design for assessment of bioequivalence test between test and reference formulations of ITZ. ITZ was given to healthy volunteers after having obtained their informed consents before enrollment to the study. Participants had not taken medications (including over-the-counter) 2 weeks prior to or during the study period. The study was performed according to the revised Declaration of Helsinki (Hyder 2001) for biomedical research involving human subjects and the rules of good clinical practice (GCP). Subjects were informed of the aim and risks of the study by the clinical investigator; based on this, they provided written informed consent before participating in the study. In addition, the Institutional Review Board (IRB) of Hanyang University Medical Center approved the protocol prior to the start of the study. 24 volunteers aged between 19 and 25 years (22.6 ± 1.7 years, M/F = 20 : 4), with body weights between 49.5 and 75.0 kg (64.8 ± 7.2 kg) and with heights between 155.0 and 181.0 cm (172.2 ± 6.5 cm) were included in this study with a 1-week washout period between doses. The participants were non-alcoholic and free from diseases. Their health status was assessed by clinical evaluation including physical examination and the following laboratory tests; albumin, alkaline phosphatase, ALT, AST, blood glucose, creatinine, blood urea nitrogen, total cholesterol, protein, total bilirubin, hemoglobin, hematocrit, total and differential white cell counts and routine urinanalysis. During each period, the volunteers were hospitalized in the clinical pharmacokinetic laboratory in Hanyang University Medical Center at 18 : 00 pm and had an evening meal before 20 : 00 pm. After fasting overnight, they received 200 mg ITZ of each formulation of the test (one 200 mg tablet) or reference (two 100 mg capsules) drugs at 7 : 00 am along with 240 ml of water. Subjects were then in the seated position for at least 1 h and then fasted for additional 4 h. A standard lunch and evening meal were provided at 4 and 10 h after dosing. Liquid consumption was allowed *ad libitum* after regular lunch except for liquids that contained xanthine or acidic beverages including tea, coffee and cola. At 0, 4, 8, 12, 24, 48, 72 h after dose, blood pressure, heart rate and body temperature were recorded. Blood samples (8 mL) were withdrawn by indwelling catheter into heparin-containing tubes from a suitable forearm vein before and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h after dosing. The blood samples were centrifuged at 3000 *g* for 10 min at room temperature and plasma was stored at $-70\,^{\circ}C$ until analysis. The total plasma ITZ concentrations were determined as the mean of duplicate samples. The maximal concentration (C_{max}) and time for maximal concentrations (T_{max}) were determined by visual inspection from each subject's plasma concentration versus time plots for ITZ. The area under the plasma concentration versus time curve (AUC_{36h}) of ITZ was calculated by the linear trapezoidal rule from 0 to 72 h. Plasma elimination half-life($T_{1/2}$, h) was calculated as $\ln 2/K_e$. The AUC extrapolated to infinity (AUC_∞) was calculated as $AUC_t + C_t/K_e$, where C_t is the last measurable concentration and the elimination constant (K_e) was obtained from the least square fitted terminal log-linear portion of the plasma concentration vs time curves (Chen et al. 2001; Shargel et al. 2005).

Acknowledgement: This work was supported by the cluster research fund of Hanyang University (HY-2007-C)

References

Ackermann B, Berna Mj, Murphy A (2002) Recent advances in use of LC/MS/MS for quantitative high-throughput bioanalytical support of drug discovery. Curr Top Med Chem 2: 53–66.

Al-Rawithi S, Hussein R, Al-Moshen I, Raines D (2001) Expedient microdetermination of itraconazole and hydroxyitraconazole in plasma by high-performance liquid chromatography with fluorescence detection. Ther Drug Monitor 23: 445–448.

Barone J, Moskovitz B, Guarnieri J, Hassell A, Colaizzi J, Bierman R, Jessen L (1998) Enhanced bioavailability of itraconazole in hydroxypropyl-beta-cyclodextrin solution versus capsules in healthy volunteers. Antimicrob Agents Chemother 42: 1862–1865.

Carrier A, Parent J (2000) Liquid chromatographic-mass spectrometric determination of itraconazole and its major metabolite, hydroxyitraconazole, in dog plasma. J Chromatogr B Biomed Sci Appl 745: 413–420.

Chen Ml, Shah V, Patnaik R, Adams W, Hussain A, Conner D, Mehta M, Malinowski H, Lazor J, Huang Sm, Hare D, Lesko L, Sporn D, Williams R (2001) Bioavailability and bioequivalence: an FDA regulatory overview. Pharm Res 18: 1645–1650.

Conway S, Etherington C, Peckham D, Brownlee K, Whitehead A, Cunliffe H (2004) Pharmacokinetics and safety of itraconazole in patients with cystic fibrosis. J Antimicrob Chemother 53: 841–847.

Guidance for industry (2001) Statistical approaches to establishing bioequivalence. USDHHS, FDA, CDER, CVM

Gubbins P, Gurley B, Bowman J (1998) Rapid and sensitive high performance liquid chromatographic method for the determination of itraconazole and its hydroxy-metabolite in human serum. Journal of pharmaceutical and biomedical analysis 16: 1005–1012.

Hardin T, Graybill J, Fetchick R, Woestenborghs R, Rinaldi M, Kuhn J (1988) Pharmacokinetics of itraconazole following oral administration to normal volunteers. Antimicrob Agents Chemother 32: 1310–1313.

Hyder A (2001) Revised declaration of Helsinki. Ethics is not just for ethicists. BMJ Clinical research ed. 323: 283; author reply 284.

Jemal M (2000) High-throughput quantitative bioanalysis by LC/MS/MS. Biomed Chromatogr 14: 422–429.

Kauffman C (1996) Role of azoles in antifungal therapy. Clin Infect Dis 22 Suppl 2: S148–153.

Koks C, Sparidans R, Lucassen G, Crommentuyn K, Beijnen J (2002) Selective high-performance liquid chromatographic assay for itraconazole and hydroxyitraconazole in plasma from human immunodeficiency virus-infected patients. J Chromatogr B Analyt Technol Biomed Life Sci 767: 103–110.

Kousoulos C, Tsatsou G, Apostolou C, Dotsikas Y, Loukas Y (2006) Development of a high-throughput method for the determination of itraconazole and its hydroxy metabolite in human plasma, employing automated liquid-liquid extraction based on 96-well format plates and LC/MS/MS. Anal Bioanal Chem 384: 199–207.

Lohitnavy M, Lohitnavy O, Thangkeattiyanon O, Srichai W (2005) Reduced oral itraconazole bioavailability by antacid suspension. J Clin Pharm Ther 30: 201–206.

Lyman C, Walsh T (1992) Systemically administered antifungal agents. A review of their clinical pharmacology and therapeutic applications. Drugs 44: 9–35.

Srivatsan V, Dasgupta A, Kale P, Datla R, Soni D, Patel M, Patel R, Mavadhiya C (2004) Simultaneous determination of itraconazole and hydroxyitraconazole in human plasma by high-performance liquid chromatography. J Chromatogr 1031: 307–313.

Suarez-Kurtz G, Bozza F, Vicente F, Ponte C, Struchiner C (1999) Limited-sampling strategy models for itraconazole and hydroxy-itraconazole based on data from a bioequivalence study. Antimicrob Agents Chemother 43: 134–140.

Uno T, Shimizu M, Sugawaeak, Tateishi T (2006) Sensitive determination of itraconazole and its active metabolite in human plasma by column-swiching high-performance liquid chromatography with ultraviolet detection. Ther Drug Monit 28: 526–531.

Vogeser M, Spohrer U, Schiel X (2003) Determination of itraconazole and hydroxyitraconazole in plasma by use of liquid chromatography-tandem mass spectrometry with on-line solid-phase extraction. Clin Chem Lab Med 41: 915–920.

Warnock D, Turner A, Burke J (1988) Comparison of high performance liquid chromatographic and microbiological methods for determination of itraconazole. J Antimicrob Chemother 21: 93–100.

Wong J, Nisar U, Yuen K (2003) Liquid chromatographic method for the determination of plasma itraconazole and its metabolite in pharmacokinetic/bioavailability studies. J Cromatogr B 798: 355–360.

Xu R, Fan L, Rieser M, El-Shourbagy T (2007) Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS. J Pharm Biomed Anal 44: 342–355.

Yao M, Chen L, Srinivas NR (2001) Quantitation of itraconazole in rat heparinized plasma by liquid chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 752: 9–16.

Yeates R, Zimmermann T, Laufen H, Albrecht M, Wildfeuer A (1995) Comparative pharmacokinetics of fluconazole and of itraconazole in Japanese and in German subjects. Int J Clin Pharmacol Therap 33: 131–135.

Yun H, Baek M, Park I, Choi B, Kwon K (2006) Comparative analysis of the effects of rice and bread meals on bioavailability of itraconazole using NONMEM in healthy volunteers. Eur J Clin Pharmacol 62: 1033–1039.