Absorption, First-Pass Metabolism, and Disposition of Itraconazole in Rats

Sun Dong YOO,* Eunhee KANG, Hun JUN, Beom Soo SHIN, Kang Choon LEE, and Kyu Hyun LEE

College of Pharmacy, Sungkyunkwan University, 300 Chonchon-dong, Jangan-ku, Suwon, Kyunggi-do, 440–746, Korea.
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This study examined the pharmacokinetic disposition, oral absorption and hepatic extraction of itraconazole and its active metabolite, hydroxyitraconazole, in rats. After i.v. injection, serum itraconazole concentrations decreased biexponentially, with an average terminal elimination half-life, volume of distribution and systemic clearance of 4.9 h, 6.0 l/kg and 14.2 ml/min/kg, respectively. When given orally, its absorption was low, with a mean absolute bioavailability of 16.6%. The metabolite to parent drug area under the curve (*AUC*) ratio was higher after oral administration compared with i.v. injection (mean ratio, 2.7 *vs.* 0.9). The hepatic drug extraction ratio determined after femoral and portal vein administration averaged 18.5%. When hydroxyitraconazole was injected i.v., the elimination half-life, volume of distribution and systemic clearance of itraconazole averaged 10.0 h, 2.4 l/kg and 3.4 ml/min/kg, respectively. The fraction of the systemically available itraconazole that was metabolized to hydroxyitraconazole was 21.0% and 76.0% after i.v. and oral administration, respectively. In summary, this study is the first reporting the hepatic extraction of itraconazole and the i.v. disposition characteristics of hydroxyitraconazole in rats. Itraconazole is a drug with a low hepatic extraction ratio and its systemic clearance appears to be largely accounted for by hepatic metabolism.

Key words itraconazole; hydroxyitraconazole; hepatic first-pass metabolism; bioavailability; metabolite kinetics

Itraconazole is an orally active triazole antifungal agent indicated for the treatment of blascomycosis, histoplasmosis, aspergillosis, oropharynea and esophageal candidiases. It is a weak base with a pK_a of 3.7 and a low aqueous solubility ($<5\ \mu$g/ml).[1)] Studies have shown that the dissolution and absorption of itraconazole is pH-dependent, with higher serum levels being obtained at lower gastric pH levels.[2)] When taken with food, its oral absorption is enhanced due to a reduced gastric pH and increased gastric emptying time.[3,4)] Alternatively, administration with gastric acid-suppressants reduces the oral bioavailability of itraconazole and this reduction can be counteracted by ingestion of acidic cola beverages.[2)] Being lipophilic (o/w partition coefficient 5.66), itraconazole is distributed extensively to tissues and its tissue levels are many times higher than in plasma.[5)] It is extensively biotransformed to more than 30 metabolites, with hydroxyitraconazole being the major active metabolite in human and rats.[1,6,7)] There are a number of publications reporting the oral absorption kinetics of itraconazole in healthy volunteers[8—10)] and in disease states,[11—13)] as well as interactions with food[3,4,14—16)] and other drugs.[17,18)] Itraconazole is a potent inhibitor of CYP3A and, when given concomitantly with other drugs such as quinidine,[19)] midazolam,[20)] bromperidol,[21)] oxybutynin,[22)] and methylprednisolone[23)] that are metabolized by this enzyme system, can significantly increase their serum levels. To our knowledge, no information has been published on the hepatic extraction of itraconazole and the i.v. disposition characteristics of hydroxyitraconazole in human and animals.

The present study was conducted to characterize the hepatic first-pass metabolism of itraconazole and the i.v. pharmacokinetics of hydroxyitraconazole in rats. The absorption kinetics, oral bioavailability and disposition of itraconazole were further investigated.

Experimental

Chemicals Acetonitrile, methylene chloride and methanol (HPLC grades) were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Ketamine, xylazine, diethylamine, *tert*-butyl methyl ether and acetic acid were obtained from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Polyethylene glycol (PEG) 400 was purchased from Yakuri Pure Chem. Co. Ltd. (Osaka, Japan). Itraconazole, hydroxyitraconazole and ketoconazole were synthesized at Choongwae Pharma Co. and were used without further purification (purity >99.2%). All other chemicals used in the study were of analytical grade.

Animals Male Sprague Dawley rats (7—9 weeks of age, 250—325 g, SPF) were obtained from Japan SLC Inc. (Shizuoka, Japan). The rats were kept in plastic rat cages and housed in an animal facility (temperature 23±2 °C) with a light/dark cycle of 12/12 h and relative humidity of 50±10%. The animals were fed with standard rat diet (DaeJong Co., Seoul, Korea) and had free access to water. At least one week of acclimatization was allowed prior to experimentation.

I.v. Injection Study Rats were anesthetized with an i.m. injection of ketamine and xylazine (90/10 mg/kg) and then cannulated with PE tubing (0.58 mm i.d. and 0.96 mm o.d., Natsume Co., Tokyo, Japan) in the right jugular and left femoral vein. Itraconazole and hydroxyitraconazole dissolved in PEG 400 (2 mg/ml) were injected into the femoral vein (5 mg/kg doses) in two groups of rats ($n=4$ each). Serial blood samples (approximately 0.3 ml each) were taken *via* the jugular vein catheter at 0, 5, 10, 15, 30 and 45 min, and 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 h after dosing. Equal volumes of saline were given as a replacement after each sampling. Serum samples were obtained by centrifugation at 1500 ***g*** for 10 min and kept at −20 °C until drug analysis.

Oral Absorption Study Rats were anesthetized and cannulated with PE tubing in the right jugular vein ($n=6$). After surgery, at least a 2 d recovery period was allowed prior to drug administration. Two mini-capsules filled with the contents of Sporanox® capsules were administered orally (itraconazole 2.5 mg per mini-capsule) with the aid of a mini-capsule injector (Natsume Co.). Immediately after capsule administration, 0.4 ml distilled water was given to facilitate swallowing. The mini-capsules had an external diameter of 2.55 mm, length 7.3 mm and a minimum capacity of 30 mm^2 (Natsume Co.). Serial blood samples (approximately 0.3 ml each) were taken from the jugular vein at 0, 5, 10, 15, 30 and 45 min, and 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 h after dosing. Equal volumes of saline were replaced after each sampling. Serum samples were obtained by centrifugation at 1500 ***g*** for 10 min and kept at −20 °C until drug analysis.

Hepatic Extraction Study Rats were anesthetized with an i.m. injection of ketamine and xylazine and were cannulated with PE tubing in the femoral artery and the portal vein or in the femoral artery and vein. Itraconazole dissolved in PEG 400 was administered *via* the femoral or portal vein on separate occasions in two groups of animals ($n=3$ each). The drug was given by simultaneous bolus injection (0.57 mg/kg) followed by infusion (0.26 mg/h/kg) for 90 min in both studies. Serial blood samples (approximately

* To whom correspondence should be addressed. e-mail: sdyoo@skku.ac.kr

0.3 ml each) were taken from the femoral artery at 0, 30, 60 and 90 min. Serum samples were obtained by centrifugation at 1500 ***g*** for 10 min and kept at −20 °C until drug analysis.

Drug Analysis Serum concentrations of itraconazole and hydroxyitraconazole were assayed by a validated HPLC method. Briefly, to 100 μl serum in borosilicate tubes (Scientific Glass Inc., Rockwood, TN, U.S.A.) was added 10 μl internal standard solution (ketoconazole 15 μg/ml in mobile phase) and 100 μl 1 M carbonate buffer (pH 10) and the mixture was mixed on a vortex mixer for 10 s. The mixture was extracted with 2 ml of *tert*-butyl methyl ether on a vortex mixer for 70 s and centrifuged at 4000 ***g*** for 10 min. The resulting supernatant was transferred to a fresh tube and dried at 45 °C under nitrogen gas. The residue was reconstituted with 125 μl mobile phase on a vortex mixer for 90 s and the reconstituted solution was centrifuged at 1500 ***g*** for 30 s and a portion (40 μl) was injected onto the chromatograph. Chromatographic separations were achieved on a Hewlett Packard 1100 series HPLC (Hewlett Packard, Santa Clara, CA, U.S.A.) using a Lichrospher 100 RP 8 (4.0×250 mm, 5 μm, Merck, Darmstadt, Germany) and a guard column (4.0×4.0 mm, 5 μm, Hewlett Packard). The mobile phase consisted of acetonitrile : 0.05% diethylamine in deionized water (6 : 4, v/v) (Milli Q Plus System, Millipore, Milford, MA, U.S.A.), with the pH adjusted to 6.0 by drop-wise addition of 30% acetic acid. The mobile phase was filtered and degassed by ultra sonication under vacuum before use. The flow rate of the mobile phase was maintained at 2.0 ml/min at ambient temperature and the effluent was monitored at a UV detection wavelength of 263 nm. Itraconazole, hydroxyitraconazole and ketoconazole (internal standard) were eluted with retention times of 2.8, 3.3 and 5.4 min, respectively. The standard curves were linear over the concentration range 10—2000 ng/ml, with a typical correlation coefficient of $r=0.9995$. The extraction recovery was >87% and >93% for itraconazole and hydroxyitraconazole, respectively, and the intra- and inter-day assay variability was <5.0% and <2.1% for itraconazole and hydroxyitraconazole, respectively, over the concentration range studied.

Data Analysis Serum itraconazole and hydroxyitraconazole concentration *vs*. time data obtained after i.v. and oral administration were analyzed by compartmental and noncompartmental methods, respectively, using the nonlinear least squares regression program WinNonlin (Scientific Consulting Inc., Cary, NC, U.S.A.). The absolute oral bioavailability of itraconazole was determined as the ratio of $AUC_{oral} \cdot D_{i.v.}/AUC_{i.v.} \cdot D_{oral}$, where the AUCs were the mean values of two groups. The steady-state drug levels after femoral and portal venous infusions were calculated as the mean of the drug concentrations determined at 30, 60 and 90 min. Pharmacokinetic parameters were expressed as the mean±S.D. Statistical differences in the pharmacokinetic parameters of itraconazole and hydroxyitraconazole after their respective i.v. injections were tested by the unpaired Student's *t*-test ($p<0.05$).

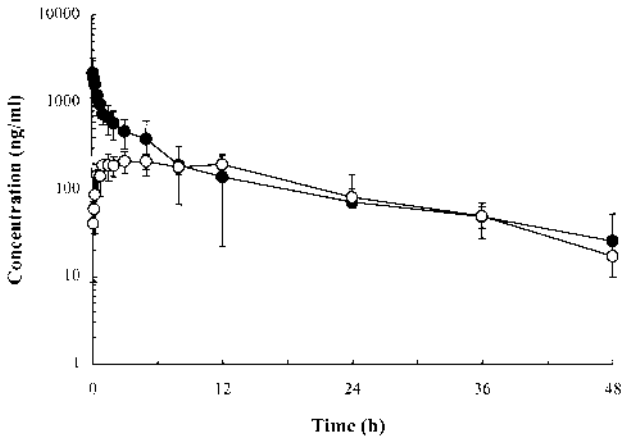

Fig. 1. Average Serum Concentration *vs*. Time Curves of Itraconazole (●) and Hydroxyitraconazole (○) after i.v Injection of Itraconazole (5 mg/kg Doses, $n=4$)

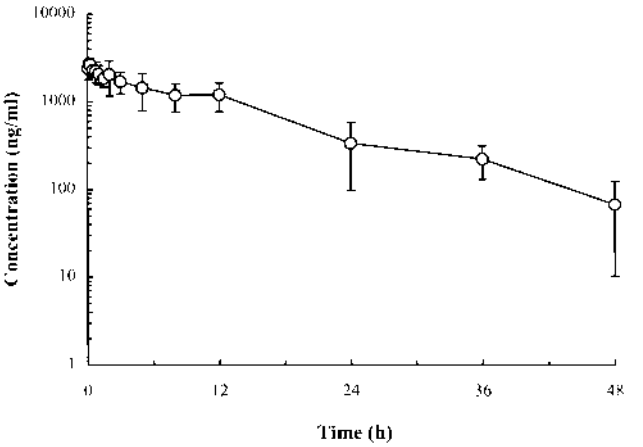

Fig. 2. Average Serum Concentration *vs*. Time Curves of Hydroxyitraconazole after i.v. Injection (5 mg/kg Doses, $n=4$)

Results and Discussion

The average serum concentration *vs*. time curves of itraconazole and hydroxyitraconazole after their respective i.v. injections are shown in Figs. 1, 2. The serum concentrations of itraconazole declined biphasically, with mean initial distribution and elimination half-lives of 0.2 and 4.9 h, respectively (Table 1). The elimination half-life of itraconazole was significantly shorter than that of hydroxyitraconazole (4.9 *vs*. 10.0 h) following i.v. injection. Consistent with its lipophilic nature (o/w partition ratio 5.66),[1)] itraconazole exhibited a large distribution volume (V_{ss} 6.0±2.5 l/kg). The mean systemic clearance of itraconazole was 14.2±7.6 ml/min/kg and the metabolite-to-parent drug *AUC* ratio (AUC_M/AUC_D) averaged 0.9±0.5. A significant correlation ($r=0.999$) was found between the systemic clearance of itraconazole and the AUC_M/AUC_D ratio in individual rats. This correlation indicates that the variations seen in the systemic clearance of itraconazole may be due to differences in the formation of hydroxyitraconazole. The elimination half-life of hydroxyitraconazole (10.0±3.4 h) after i.v. injection was comparable with that (12.4±2.0 h) found after administration of the parent drug. The systemic clearance (3.4±2.0 ml/min/kg) and distribution volume (2.4±0.7 l/kg) of hydroxyitraconazole were less than that of the parent drug. The fraction of hydroxyitraconazole formed after i.v. injection of the parent drug, calculated as $CL_M \cdot AUC_M/CL_D \cdot AUC_D$, was 21.0% of the administered dose.

Table 1. Pharmacokinetic Parameters (Mean±S.D.) Obtained after i.v. Injection of Itraconazole and Hydroxyitraconazole in Rats (5 mg/kg Doses)

Parameter	Drug i.v.		Metabolite i.v.
	Itraconazole	Hydroxyitraconazole	Hydroxyitraconazole
Weight (g)	318±30	318±30	320±38
$t_{1/2,\lambda1}$ (h)	0.2±0.1	—	0.6±0.5
$t_{1/2,\lambda z}$ (h)	4.9±5.6	12.4±2.0	10.0±3.4
C_{max} (ng/ml)	—	254±24	—
T_{max} (h)	—	12.0±5.4	—
MRT (h)	8.8±4.9	19.1±1.7	14.0±4.7
AUC (ng/h/ml)	7385±3991	5523±204	31279±14289
AUMC (ng/h/ml)	78560±76167	105717±11689	477923±305789
CL (ml/min/kg)	14.2±7.6	—	3.4±2.0
V_c (l/kg)	1.9±1.0	—	1.9±0.5
V_{ss} (l/kg)	6.0±2.5	—	2.4±0.7

Figure 3 shows the average serum concentration *vs*. time curves of itraconazole and hydroxyitraconazole after oral administration of itraconazole (5 mg doses). Itraconazole was

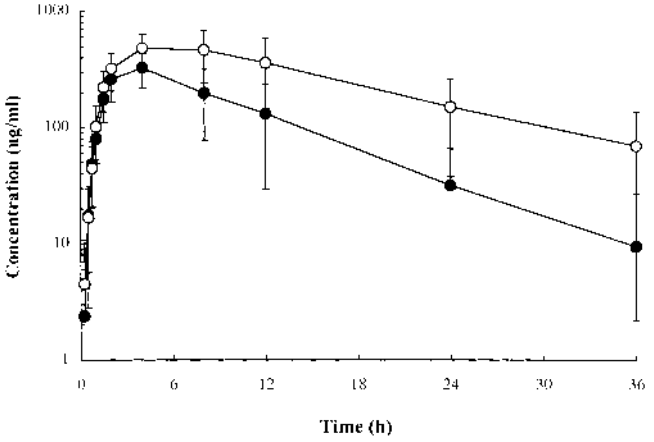

Fig. 3. Average Serum Concentration *vs*. Time Curves of Itraconazole (●) and Hydroxyitraconazole (○) after Oral Administration of 50 mg Doses of Itraconazole ($n=6$)

Table 2. Pharmacokinetic Parameters (Mean±S.D.) of Itraconazole and Hydroxyitraconazole after Oral Administration of Itraconazole (5 mg Doses) in Rats

Parameter	Itraconazole	Hydroxyitraconazole
Weight (g)	317±29	317±29
$t_{1/2,\lambda z}$ (h)	6.0±2.3	10.4±2.8
C_{max} (ng/ml)	328±107	527±197
T_{max} (h)	4.0±1.1	6.7±3.3
MRT (h)	9.7±3.1	16.4±4.9
AUC (ng/h/ml)	3864±2381	10178±5807
AUMC (ng/h^2/ml)	42280±37856	181719±147702
CL/F (ml/min/kg)	89.4±47.3	—
V_z/F (l/kg)	40.7±16.9	—

slowly absorbed, with a T_{max} being reached at 4.0 and 6.7 h for the parent drug and metabolite, respectively (Table 2). Serum levels of the metabolite exceeded that of the parent drug from approximately 2 h after administration and remained elevated thereafter. The peak concentrations (C_{max}) were higher for the metabolite than for the parent drug (mean 527 *vs.* 328 ng/ml). The apparent elimination half-lives of the drug and metabolite (6.0 and 10.4 h, respectively) were comparable with those (4.9 and 10.0 h, respectively) after i.v. injection (Table 1). These elimination half-lives of itraconazole are comparable with that reported in rats previously (6.5 h).[1] The mean AUC_M/AUC_D ratio after oral administration was higher than that after i.v. injection (2.7 *vs*. 0.9). The absolute bioavailability of itraconazole was low (16.6%) and most (76.0%) of the absorbed drug was converted to hydroxyitraconazole, as calculated from the drug and metabolite *AUC* and clearance values. In human, the relative oral bioavailability of itraconazole from capsules over solution is 39.8%[3] and the absolute oral bioavailability from solution is 55%.[7] It is not known whether the low oral bioavailability of itraconazole in rats and human is due to poor dissolution and absorption, hepatic first-pass metabolism or extensive gut wall metabolism.

The extent of hepatic drug extraction was determined by comparing the arterial serum drug levels after separate femoral and portal venous infusions[24] of itraconazole. There were no statistical differences in the arterial drug concentrations determined at 30, 60 and 90 min, indicating that steady-state was achieved within 30 min of infusion. The mean steady-state hepatic extraction of itraconazole averaged 18.5%. The systemic clearance of itraconazole obtained after femoral venous infusion averaged 15.5 ml/min/kg, which was comparable with that determined in the i.v. bolus injection study (14.2 ml/min/kg). Assuming an even distribution of itraconazole between serum and erythrocytes, its systemic clearance accounts for <30% of the hepatic blood flow (55.2 ml/min/kg) in rats.[25] Therefore, the hepatic clearance of itraconazole (10.5 ml/min/kg), calculated as the product of the hepatic blood flow and hepatic extraction ratio, appears to account for most of its systemic clearance.

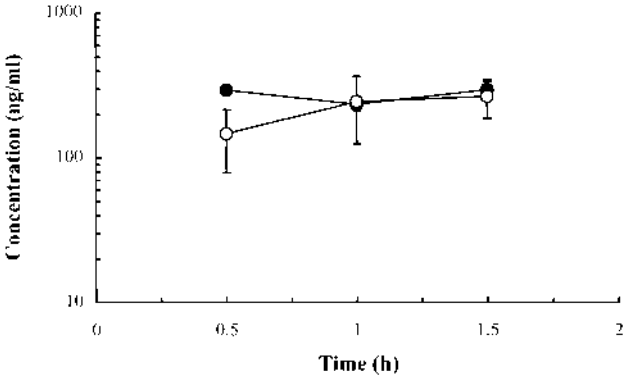

Fig. 4. Average Arterial Serum Concentrations of Itraconazole Following Simultaneous i.v. Bolus Injection (0.57 mg/kg) Plus Infusion (0.26 mg/h/kg) into the Femoral Vein (●) and the Portal Vein (○) on Separate Occasions ($n=3$ each)

Summary and Conclusions

This study examined the pharmacokinetic disposition of itraconazole and hydroxyitraconazole in rats. Itraconazole exhibited a greater systemic clearance (14.2 *vs*. 3.4 ml/min/kg), greater steady-state volume of distribution (6.0 *vs*. 2.4 l/kg) and shorter elimination half-life (4.9 *vs*. 10.0 h) compared with hydroxyitraconazole. The absolute oral bioavailability (16.6%) and hepatic extraction (18.5%) of itraconazole were low. Nevertheless, the systemic clearance of itraconazole appears to be largely due to its hepatic clearance.

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