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Interspecies Comparison of the Oral Absorption of Itraconazole in Laboratory Animals

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The oral absorption and disposition of itraconazole were studied in rats, rabbits and dogs. Serum levels of itraconazole and its active metabolite, hydroxyitraconazole, were determined by a validated HPLC method. The absorption of itraconazole was relatively rapid in rats and dogs but was slower in rabbits. The terminal elimination half-life ($T_{1/2,\lambda z}$), time to the peak concentration (T_{max}), dose and weight normalized area under the curve (AUC) and the peak concentration (C_{max}) of itraconazole found in the dog were comparable to those reported in humans. As in humans, the metabolite to parent drug AUC ratios in rats and dogs were greater than unity but was less in rabbits. The dog appears to be an appropriate animal model while the rat, not the rabbit, may be used as an alternative animal model in predicting the oral absorption of itraconazole in humans.

Key words: Itraconazole, Pharmacokinetics, Absorption, Hydroxyitraconazole

INTRODUCTION

Itraconazole is an orally active, systemic triazole antifungal agent indicated in the treatment of blastomycosis, histoplasmosis, aspergillosis, oropharyngeal and esophageal candidiases. It is a weakly basic drug (pKa 3.7, m.w. 705.6), with high lipophilicity (log o/w partition coefficient 5.66), and is soluble only under extremely acidic conditions (Heykants *et al.*, 1987). Upon administration, the gastric acidity is required for an adequate dissolution and absorption of itraconazole in the gastrointestinal tract (Haria *et al.*, 1996). The absolute oral bioavailaility of itraconazole in solution is 55% (Heykants *et al.*, 1989). Reduced absorption is observed when administered in the fasting state, with AUC and C_{max} being approximately 60% of those obtained after food (Grant and Clissold, 1989; Van Peer *et al.*, 1989; Barone *et al.*, 1993). Itraconazole exhibits a large volume of distribution and high protein binding (< 99%) (Heykants *et al.*, 1987, 1990). In humans and animals, it is biotransformed to more than 30 metabolites. Of these, hydroxyitraconazole is the major active metabolite possessing the antifungal activity similar to that of the parent drug *in vitro* (Heykants *et al.*, 1987; Dupont and Drouhet, 1987). Limited information is available on the metabolism of itraconazole in animals and humans (Heykants *et al.*, 1987; Lange *et al.*, 1997; Yoo *et al.*, 2000).

Despite extensive pharmacokinetic studies in humans, only few studies have examined on the oral absorption kinetics of itraconazole in laboratory animals (Heykants et al., 1987; Yoo et al., 2000). Furthermore, no discussion has been made in the literature on the suitability of animal models useful in the preclinical evaluation of oral dosage forms. Therefore, the purpose of this study was to evaluate the oral absorption and disposition of itraconazole in several laboratory animals such as rats, rabbits and dogs. The pharmacokinetic parameters of itraconazole and hydroxyitraconazole obtained in these animals were compared with those reported in humans. Further, the suitability of these laboratory animals as a model to study the oral absorption kinetics of itraconazole was discussed.

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MATERIALS AND METHOD

Chemicals

Acetonitrile, methylene chloride and methanol (HPLC grades) were purchased from J.T. Baker (Phillipsburg, NJ). Ketamine, xylazine, diethylamine, *t*-butyl methyl ether and acetic acid were obtained from Sigma Chem. Co. (St. Louis, MO). 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) and New Zealand White rabbits (4-5 months of age, 3.0-3.5 kg) were obtained from Japan SLC Inc. (Shizuoka, Japan). The rats and rabbits were kept in plastic rat cages and stainless steel rabbit cages, respectively, and were housed in an animal facility (temperature 23 ± 2°C) with light/dark cycle of 12/12 hr and relative humidity of 50 ± 10%. The animals were fed standard rat and rabbit diet (DaeJong Co., Seoul, Korea) and had free access to water. Beagle dogs (4-6 months of age, 8.6-9.3 kg) were purchased from Jungang Animal Co. (Seoul, Korea) and maintained in stainless steel dog cages with free access to standard dog chow (DaeJong Co., Seoul, Korea) and water.

Rat Study

After at least one week of acclimatization period, the rats were anesthetized with i.m. injection of ketamine and xylazine (90/10 mg/kg) and cannulated with PE tubing (0.58 mm i.d. and 0.96 mm o.d., Natume Co., Tokyo, Japan) in the right jugular vein (n=6). After surgery, at least 2 days of recovery period was allowed prior to drug administration. Before dosing, the animals were fasted overnight (> 18 hr) and for at least 4 hr after drug administration. Two mini capsules filled with the content of Sporanox® capsules were administered orally (itraconazole 2.5 mg per mini capsule) with an aid of a mini capsule injector (Natsume Co., Tokyo, Japan) followed by ingestion of 0.4 ml of distilled water to facilitate swallowing. The mini capsules had an external diameter cap 2.55 mm, length 7.3 mm and minimum capacity 30 mm^2 (Natsume Co., Tokyo, Japan). Serial blood samples (approximately 0.3 ml each) were taken *via* the jugular vein catheter at 0, 5, 10, 15, 30 and 45 min, 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 hr after dosing. Equal volumes of saline were injected after each sampling. Serum samples were harvested by centrifugation at 1,500 *g* for 10 min and were kept at -20°C until drug analysis.

Rabbit Study

Prior to drug administration, the rabbits (n=4) were kept collared to prevent coprophargy and fasted overnight (> 12 hr), with the fasting continued for at least 4 hr after administration. Capsules filled with the content of Sporanox® capsules (itraconazole 50 mg doses) were administered orally followed by ingestion of 3 ml of water to facilitate swallowing. Serial blood samples (approximately 0.5 ml each) were collected from the marginal ear vein at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 hr after dosing. Serum samples harvested by centrifugation at 1,500 *g* for 10 min were kept at -20°C until drug analysis.

Beagle Dog Study

After overnight fasting (> 18 hr), Beagle dogs (n=4) were administered with Sporanox® capsules (itraconazole 100 mg doses) followed by ingestion of 10 ml of distilled water to facilitate swallowing. Food was not given for at least 4 hr after drug administration. Serial blood samples (approximately 0.5 ml each) were taken from the lateral tarsal vein at 0, 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 24, 36, 48, 72 and 96 hr after dosing. Serum samples were obtained by centrifugation at 1,500 *g* for 10 min and were kept at -20°C until analysis.

Drug Analysis

Serum concentrations of itraconazole and hydroxyitraconazole were determined by a validated HPLC method. Briefly, to an aliquot (100 μl) of the serum sample in borosilicate tubes were added 10 μl of the internal standard solution (ketoconazole 15 μg/ml in mobile phase) and 100 μl of 1 M carbonate buffer (pH 10). The mixture was extracted with 2 ml of *t*-butyl methyl ether on a vortex mixer for 70 sec and centrifuged at 4,000 *g* for 10 min. The resulting supernatant was transferred into a fresh tube and dried at 45°C under nitrogen gas. The residue was reconstituted with 125 μl of the mobile phase on a vortex mixer for 90 sec. The reconstituted solution was centrifuged at 1,500 *g* for 30 sec and a portion (40 μl) was injected onto the chromatograph. The chromatographic system used in the study was a Hewlett Packard 1100 series HPLC consisting of a model G1311A quarternary pump, G1313A autosampler, G1315A diode array detector, G1316A column compartment, G1322A degasser and HP Chem Station (Ver. 4.02) chromatography manager software (Hewlett Packard, Santa

Clara CA). Chromatographic separations were achieved using a Lichrospher 100 RP 8 (Merck, 4.0 mm × 250 mm, 5 μm Darmstadt, Germany) and a guard column (HP, 4.0 mm × 4.0 mm, 5 μm, Santa Clara, CA). The mobile phase consisted of acetonitrile:0.05% diethylamine in deionized water (6:4, v/v) (Milli Q Plus System, Millipore, Milford, MA) with pH adjusted to 6.0 by 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 the retention times of 2.8, 3.3 and 5.4 min, respectively. The standard curve was linear over the concentration range of 10-2,000 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 coefficients of variation were < 5.0% and < 2.1% for itraconazole and hydroxyitraconazole, respectively, over the dose range studied (n=4 at each concentration for the rat, rabbit and dog sera).

Data Analysis

Serum itraconazole and hydroxyitraconazole concentration *vs.* time data were analyzed by a noncompartmental method using WinNonlin (Scientific Consulting Inc., Cary, NC). Pharmacokinetic parameter values were expressed as the mean ± S.D. The statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Average serum concentration *vs.* time curves of itraconazole and hydroxyitraconazole obtained in rats, rabbits and dogs are shown in Fig. 1-3, respectively. Pharmacokinetic parameters of the parent drug and the metabolite are reported in Tables 1 and 2, respectively. As in humans (Hardin *et al.*, 1988; Zimmermann *et al.*, 1994), large inter-individual variations were observed in the pharmacokinetic parameters of itraconazole in these animals. The absorption of itraconazole was relatively rapid in rats and dogs compared to rabbits. The T_{max} of itraconazole was greater in rabbits (8.7 ± 3.0 hr) than in rats (5.2 ± 3.6 hr), dogs (3.3 ± 2.1 hr) and humans (3.0-3.9 hr) determined in the fasting state (Van Peer *et al.*, 1989; Barone *et al.*, 1993; Zimmermann *et al.*, 1994). The mean terminal elimination half-life of itraconazole in the dog (28.0 ± 2.9 hr) was comparable to that found in healthy humans (range 21-24 hr) (Van Peer *et al.*, 1989; Barone *et al.*, 1993; Lange *et al.*, 1997) but was longer than those found in rats (5.2 ± 1.8 hr) and rabbits (9.4 ± 3.5 hr).

For comparison purposes, AUC and C_{max} values of itraconazole obtained in three animal species were normalized by the milligram dose per kilogram body

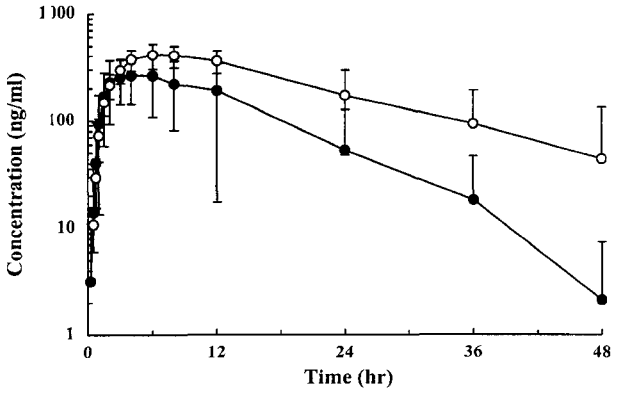

Fig. 1. Average serum concentration *vs.* time curves of itraconazole (λ) and hydroxyitraconazole (o) in rats (n=6) after administration of capsules containing 5 mg doses of itraconazole.

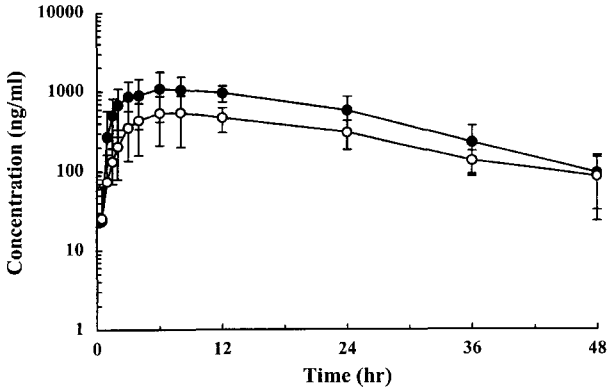

Fig. 2. Average serum concentration *vs.* time curves of itraconazole (λ) and hydroxyitraconazole (o) in rabbits (n=5) after administration of capsules containing 50 mg doses of itraconazole.

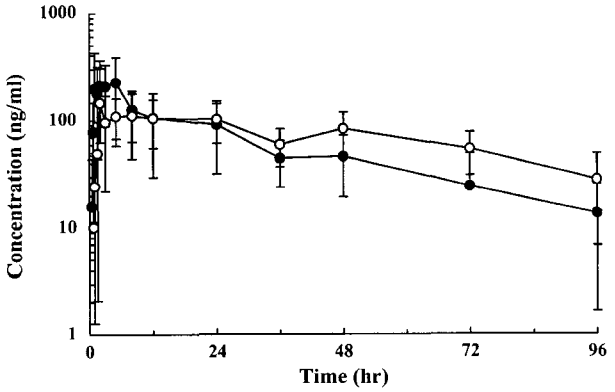

Fig. 3. Average serum concentration *vs.* time curves of itraconazole (λ) and hydroxyitraconazole (o) in Beagle dogs (n=4) after administration of capsules containing 100 mg doses of itraconazole.

Table 1. Pharmacokinetic parameters (mean ± S.D.) of itraconazole obtained after oral administration of itraconazole in the rats, rabbits and dogs at 5, 50 and 100 mg doses, respectively

Parameter	Rats (n=6)	Rabbits (n=4)	Dogs (n=4)
Weight (kg)	0.27 ± 0.02	3.4 ± 0.4	8.9 ± 0.4
AUC (μg·hr/ml)	4.6 ± 3.4	27.9 ± 11.8	6.4 ± 2.7
AUMC (μg·hr²/ml)	58.6 ± 58.8	533.9 ± 249.9	249. 7 ± 161.5
C_{max} (ng/ml)	368.0 ± 108.5	1230.8 ± 533.2	371.7 ± 125.4
T_{max} (hr)	5.2 ± 3.6	8.7 ± 3.0	3.3 ± 2.1
$T_{1/2,\lambda z}$ (hr)	5.2 ± 1.8	9.4 ± 3.5	28.0 ± 2.9
MRT_{po} (hr)	10.5 ± 4.5	18.6 ± 3.9	36.7 ± 9.8
Cl/F (L/hr)	1.6 ± 0.9	2.2 ± 1.2	18.2 ± 8.4
Cl/F (L/hr/kg)	6.0 ± 3.4	0.7 ± 0.4	2.1 ± 0.9
V_z/F (L)	10.3 ± 0.9	26.8 ± 10.0	731.9 ± 346.4
V_z/F (L/kg)	38.6 ± 3.4	7.9 ± 2.9	82.2 ± 38.9

Table 2. Pharmacokinetic parameters (mean ± S.D.) of hydroxyitraconazole obtained after oral administration of itraconazole in rats, rabbits and dogs at 5, 50 and 100 mg doses, respectively

Parameter	Rats (n=6)	Rabbits (n=4)	Dogs (n=4)
Weight (kg)	0.27 ± 0.02	3.4 ± 0.4	8.9 ± 0.4
AUC (μg·hr/ml)	10.5 ± 4.6	14.7 ± 5.7	9.4 ± 1.4
AUMC (μg·hr²/ml)	223.3 ± 162.6	319.1 ± 112.1	777.9 ± 401.1
C_{max} (ng/ml)	468.1 ± 71.5	590.0 ± 300.6	218.4 ± 103.7
T_{max} (hr)	8.0 ± 3.3	7.1 ± 3.4	3.8 ± 1.5
$T_{1/2,\lambda z}$ (hr)	11.8 ± 4.5	12.4 ± 3.1	57.5 ± 49.1
MRT (hr)	19.3 ± 6.4	22.2 ± 4.1	88.6 ± 61.0
AUC_M/AUC_D	2.7 ± 0.9	0.5 ± 0.1	1.6 ± 0.5

weight and compared with those calculated in humans under the fasting state (Van Peer *et al.*, 1989; Barone *et al.*, 1993; Lange *et al.*, 1997). The average dose- and weight-normalized AUC in the dog (0.57 μg·hr/ml) was within the range reported in humans (0.51-0.67 μg·hr/ml), while it was lower in the rat (0.25 μg·hr/ml) and higher in the rabbit (1.9 μg·hr/ml). Similarly, the average dose- and weight-normalized C_{max} in the dog (33.1 ng/ml) was comparable to those found in humans (range, 27.3-52.2 ng/ml), while it was lower in the rat (19.9 ng/ml) and higher in the rabbit (83.7 ng/ml).

There were species-specific differences in the pharmacokinetics of hydroxyitraconazole. The apparent elimination half-life of hydroxyitraconazole was significantly longer than for the parent drug in rats (11.8 ± 4.5 hr *vs.* 5.2 ± 1.8 hr) and dogs (57.5 ± 49.1 hr *vs.* 28.0 ± 2.9 hr), while in rabbits, they were comparable (12.4 ± 3.1 hr *vs.* 9.4 ± 3.5 hr) (Table 1-2). The mean metabolite to parent drug AUC ratios (AUC_M/AUC_D) in the rat and the dog were greater than unity (2.7 ± 0.9 and 1.6 ± 0.5, respectively), while it was less in rabbits (0.54 ± 0.06). The mean AUC_M/AUC_D found in rats is comparable to that reported in humans (mean ratio 2.48) (Barone *et al.*, 1993). Fig. 4 shows the relationship between AUC_M/AUC_D and the oral clearance of itraconazole (Cl/F) in these three animal species. Significant correlations were found in the rat (r^2 = 0.765) and the dog (r^2 =0.902) but not in the rabbit (r^2 =0.349) (Fig. 4). Assuming the systemic clearance of hydroxyitraconazole is relatively consistent across individual animals, the significant correlation found in rats and dogs suggest that the formation of hydroxyitraconazole may be a contributing factor in the variations seen in the oral clearance of itraconazole, while this is not the case in rabbits.

Itraconazole is soluble only under extremely acidic conditions and, therefore, the gastric pH may play an important role in its oral absorption. Alterations in the gastric pH are known to significantly influence the oral absorption of itraconazole (Lange *et al.*, 1997). The resting gastric pH in the rabbit (median pH 1.9) is similar to that reported in humans (pH 0.9-1.53) but is higher in the rat (pH 5.0) and the dog (pH 5.5) (Davies and Morris 1993; Lange *et al.*, 1997). The similarity in the gastric pH between humans and rabbits contradicts the poor prediction of the oral absorption of itraconazole in humans. This contradiction may be explained by much longer gastric emptying time in the rabbit (Chiou *et al.*, 1969; Dressman and Yamada 1991; Aoyagi *et al.*, 1992). The prolonged gastric emptying time of itraconazole in rabbits is consistent with the greater T_{max} than found in rats, dogs and humans. In humans, ingestion of food decreases the median gastric pH (from 1.35 to 0.90) and increases the gastric emptying time (from 0.50 hr to 5.59 hr), favoring the oral absorption of itraconazole (Zimmermann *et al.*, 1994). This observation suggests that both the gastric emptying time and the gastric pH may be important in the absorption of itraconazole.

In summary, there were differences in the absorption and disposition kinetics of itraconazole and the elimination of hydroxyitraconazole in rats, rabbits and dogs. The dog may be an appropriate animal model to study the oral absorption of itraconazole, given the comparable T_{max}, $T_{1/2,\lambda z}$, dose and weight normalized AUC and C_{max} between the dog and humans. Considering the easy accessibility and handling, the rat, but not the rabbit, may be an alternative animal model in studying the oral absorption of itraconazole based on similar T_{max} and AUC_M/AUC_D between humans and rats.

REFERENCES

Aoyagi, N., Ogata, H., Kaniwa, N., Uchiyama, M., Yasuda, Y. and Tanioka, Y., Gastric emptying of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled

rabbits. *J. Pharm. Sci.,* 81, 1170-1174 (1992).

Barone, J.A., Koh, J.G., Bierman, R.H., Colaizzi, J.L., Swanson, K.A., Gaffar, M.C., Moskovitz, B.L., Mechlinski, W. and Van de Velde, V., Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers. *Antimicrob. Agents Chemother.*, 37, 778-784 (1993).

Chiou, W.L., Riegelman, S. and Amberg, J.R., Complications in using rabbits for the study of oral drug absorption. *Chem. Pharm. Bull.*, 17, 2170-2173 (1969).

Davies, B. and Morris, T., Physiological parameters in laboratory animals and humans. *Pharm. Res.*, 10, 1093-1095 (1993).

Dressman, J. and Yamada, K., Animal models for oral drug absorption. In Welling, P.G., Tse, F.L.S., Dighe, S.V. (Eds.). *Pharmaceutical Bioequivalence.* Marcel Dekker Inc., New York, pp. 235-266, 1991.

Dupont, B. and Drouhet, E., Early experience with itraconazole in vitro and in patients: pharmacokinetic studies and clinical results. *Rev. Infect. Dis.*, 9, S71-S76 (1987).

Grant, S.M. and Clissold, S.P., Itraconazole. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in superficial and systemic mycoses. *Drugs,* 37, 310-344 (1989).

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

Haria, M., Bryson, H.M. and Goa, K.L., Itraconazole: a reappraisal of its pharmacological properties and therapeutic use in the management of superficial fungal infections. *Drugs,* 51, 585-620 (1996).

Heykants, J., Michiels, M., Meuldermans, W., Monbaliu, J., Lavrijsen, K., Van Peer, A., Levron, J.C., Woestenborghs, R. and Cauwenbergh, G., The pharmacokinetics of itraconazole in animals and man: an overview. In Fromtling, R.A., (Ed.). *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents.* J. R. Prous Science Publishers, Barcelona, pp. 223-249, 1987.

Heykants, J., Van Peer, A., Van de Velde, V., Van Rooy, P., Meuldermans, W., Lavrijsen, K., Woestenborghs, R., Van Cutsem, J. and Cauwenbergh, G., The clinical pharmacokinetics of itraconazole: an overview. *Mycoses,* 32, 67-87 (1989).

Heykants, J., Van Peer, A., Lavrijsen, K., Meuldermans, W., Woestenborghs, R. and Cauwenbergh, G., Pharmacokinetics of oral antifungals and their clinical implications. *Br. J. Clin. Pract. Suppl.,* 71, 50-56 (1990).

Lange, D., Pavao, J.H., Wu, J. and Klausner, M., Effect of a cola beverage on the bioavailability of itraconazole in the presence of H_2 blockers. *J. Clin. Pharmacol.*, 37, 535-540 (1997).

Van Peer, A., Woestenborghs, R., Heykants, J., Gasparini, R. and Cauwenbergh, G., The effects of food and dose on the oral systemic availability of itraconazole in healthy subjects. *Eur. J. Clin. Pharmacol.,* 36, 423-426 (1989).

Yoo, S.D., Kang, E., Jun, H., Shin, B.S., Lee, K.C. and Lee, K.H., Absorption, first-pass metabolism, and disposition of itraconazole in rats. *Chem. Pharm. Bull.*, 48, 798-801 (2000).

Zimmermann, T., Yeates, R.A., Albrecht, M., Laufen, H. and Wildfeuer, A., Influence of concomitant food intake on the gastrointestinal absorption of fluconazole and itraconazole in Japanese subjects. *Int. J. Clin. Pharmacol. Res.,* 14, 87-93 (1994).