

Pharmacokinetics of Itraconazole in Diabetic Rats[∇]

Unji Lee,¹ Young H. Choi,² So H. Kim,³ and Byung K. Lee^{1*}

College of Pharmacy, Ewha Womans University, 11-1, Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, South Korea¹; College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea²; and Department of Pharmacology, College of Dentistry and Research Institute of Oral Science, Kangnung-Wonju National University, 120, Gangneung Daehangno, Gangneung, Kyunggi-Do 210-702, South Korea³

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After intravenous or oral administration of 10 mg/kg itraconazole to rats with streptozotocin-induced diabetes mellitus and to control rats, the total area under the plasma concentration-time curve from time 0 to 24 h (AUC_{0–24}) for itraconazole and that for its metabolite, 7-hydroxyitraconazole, were similar between the two groups of rats. This may be explained by the comparable hepatic and intestinal intrinsic clearance rates for the disappearance of itraconazole and the formation of 7-hydroxyitraconazole in both groups of rats.

Itraconazole is a prototype triazole antifungal agent. Superficial fungal infections of the feet among elderly patients with diabetes mellitus are common, and itraconazole has been shown to have acceptable cure rates (12). In humans, hepatic cytochrome P450 3A4 (CYP3A4) appears to be involved in the metabolism of itraconazole to form several metabolites, including 7-hydroxyitraconazole (9). No *in vivo* studies of itraconazole metabolism in rats have been reported. Hepatic CYP3A1 (5) and CYP3A2 (10) proteins and/or mRNA levels have been shown to increase in male Sprague-Dawley rats with diabetes mellitus induced by streptozotocin (DMIS rats), but there are no reports on the intestinal CYP3A subfamily in DMIS rats. Furthermore, the pharmacokinetics of itraconazole and 7-hydroxyitraconazole may differ between intravenously and orally administered itraconazole in DMIS rats.

In the present study, itraconazole metabolism was examined in DMIS rats as an animal model of diabetes mellitus. We

report the pharmacokinetics of itraconazole and 7-hydroxyitraconazole after intravenous or oral administration in DMIS rats compared with those in control rats. Our results show that hepatic CYP3A1/2 is responsible for the metabolism of itraconazole and the formation of 7-hydroxyitraconazole in rats and that the expression of the intestinal CYP3A1/2 protein was not altered in DMIS rats compared with that in control rats, based on Western blot analysis.

Overall, the methods used in this study were similar to those described in previous reports. The chemicals used in addition to itraconazole, the methods of housing and handling the male Sprague-Dawley rats (7 to 9 weeks old, weighing 230 to 280 g), the intravenous and oral administration of itraconazole, the measurement of plasma protein binding values of itraconazole by equilibrium dialysis, and the high-performance liquid chromatographic analysis of itraconazole and 7-hydroxyitraconazole were all performed as described previously (1, 11). Dia-

TABLE 1. V_{max} , K_m , and CL_{int} values for the disappearance of itraconazole and formation of 7-hydroxyitraconazole in hepatic and intestinal microsomes from control and DMIS rats

Parameter	Values for rats ^a			
	Disappearance of itraconazole		Formation of 7-hydroxyitraconazole	
	Control (n = 4)	DMIS (n = 4)	Control (n = 4)	DMIS (n = 4)
Hepatic microsomes				
V_{max} (nmol/min/mg protein)	0.111 ± 0.0813	0.705 ± 0.505	0.00903 ± 0.00136	0.00788 ± 0.00104
K_m (μM)	11.1 ± 6.00	57.7 ± 39.1	1.69 ± 0.768	1.41 ± 0.668
CL_{int} (ml/min/mg protein)	0.00904 ± 0.00290	0.0146 ± 0.00559	0.00602 ± 0.00204	0.00660 ± 0.00310
Intestinal microsomes				
V_{max} (nmol/min/mg protein)	0.207 ± 0.155	0.124 ± 0.0861	0.00345 ± 0.00109	0.00383 ± 0.000222
K_m (μM)	48.4 ± 33.1	31.2 ± 27.5	0.240 ± 0.133	0.461 ± 0.190
CL_{int} (ml/min/mg protein)	0.00413 ± 0.000926	0.00485 ± 0.00150	0.0166 ± 0.00832	0.00965 ± 0.00467

^a Values are shown as means ± standard deviations.

* Corresponding author. Mailing address: College of Pharmacy, Ewha Womans University, 11-1, Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, South Korea. Phone: 82 2 32776649. Fax: 82 2 32772851. E-mail: bklee@ewha.ac.kr.

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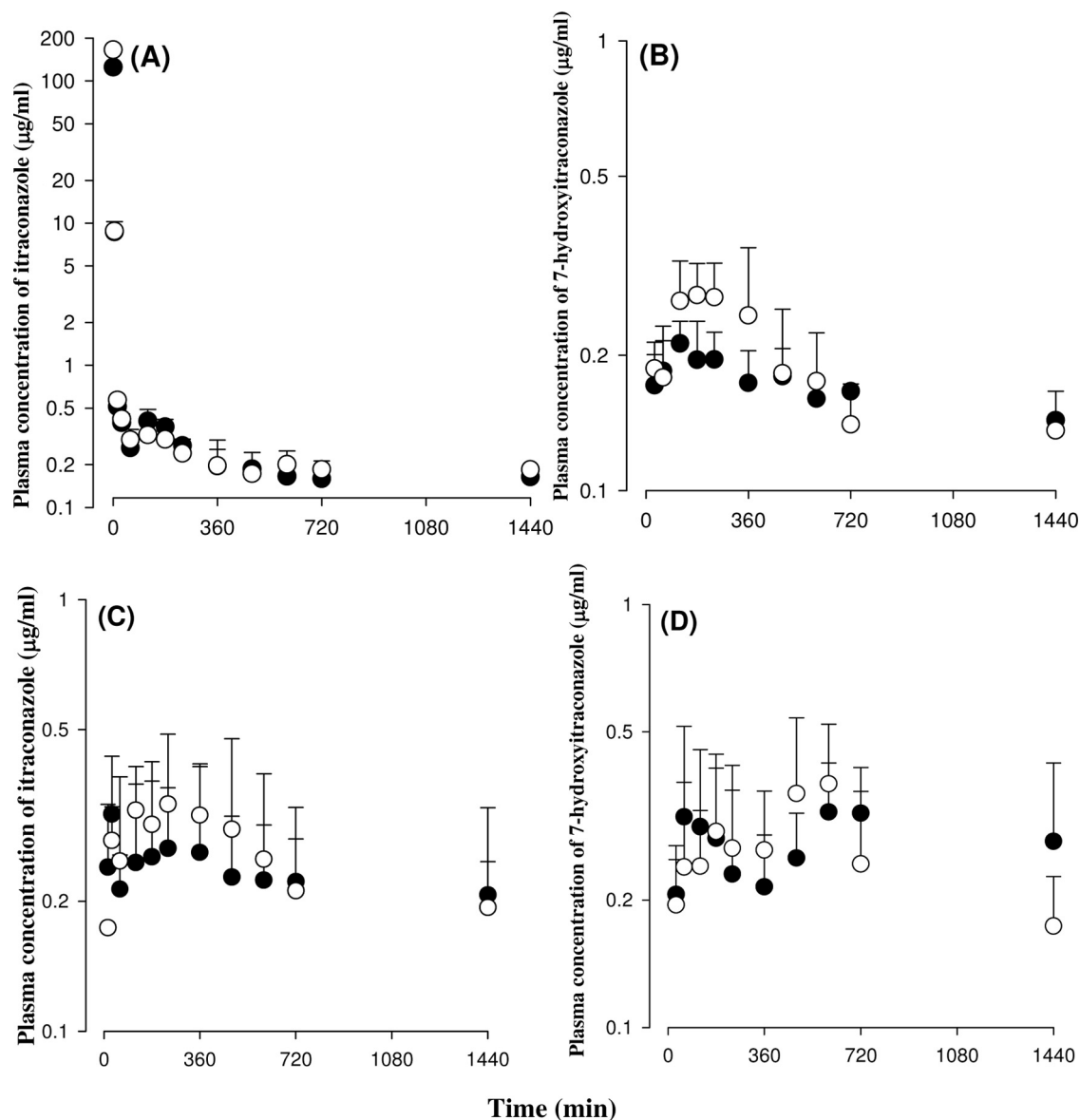


FIG. 1. Mean arterial plasma concentration-time profiles of itraconazole (A, C) and 7-hydroxyitraconazole (B, D) after intravenous (A, B) or oral (C, D) administration of itraconazole at 10 mg/kg in control (●) and DMIS (○) rats. Error bars, standard deviations.

betes mellitus was induced with streptozotocin (5). Seven control rats and eight DMIS rats were used in the intravenous administration study. Nine control rats and nine DMIS rats were used in the oral study. Intravenous administration of itraconazole to control rats pretreated with dexamethasone and troleandomycin was performed as previously described (3). Hepatic and intestinal microsomes were prepared from control and DMIS rats (6). The protein expression of intestinal CYP3A1/2 was examined by Western blot analysis (7).

The procedures for measuring V_{\max} and K_m for the disappearance of itraconazole and the formation of 7-hydroxyitraconazole were similar to those used in a previous report (6). Microsomes (equivalent to 0.5 mg protein); 5 μ l of dimethyl sulfoxide containing 2.5, 5, 10, 20, 30, or 50 μ M itraconazole; and 50 μ l of 0.1 M phosphate buffer (pH 7.4) containing 1 mM

NADPH were mixed and incubated for 0, 15, 30, 45, or 60 min for hepatic microsomes or for 5, 15, 30, 45, 60, or 75 min for intestinal microsomes. All microsomal incubation conditions were within the linear range of the reaction. After incubation for 45 min (for hepatic microsomes) or 50 min (for intestinal microsomes), 100 μ l of each reaction mixture was transferred to a test tube containing 100 μ g/ml R51012 (internal standard) in 50 μ l of acetonitrile, 250 μ l of 0.1 M carbonate buffer (pH 9.8), and 1 ml of methyl *t*-butyl ether. The kinetic constants (K_m and V_{\max}) were calculated using a nonlinear regression method (4). Intrinsic clearance (CL_{int}) was calculated by dividing V_{\max} by K_m .

The total area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) or from time 0 to the last measured time at 24 h (AUC_{0-24}) was calculated using the

trapezoidal rule-extrapolation method (2). The peak plasma concentration (C_{\max}) and time needed to reach C_{\max} (T_{\max}) were directly read from the experimental data. The percentage of the dose excreted in a 24-h urine sample (Ae_{0-24}) and that recovered from the gastrointestinal tract (including its contents and feces) sampled after 24 h (GI_{0-24}) were also measured (11). All results are expressed as mean values \pm standard deviations, with the exception of values for T_{\max} , which are expressed as median values with ranges. Unpaired t tests were performed, and P values of <0.05 were regarded as statistically significant.

Plasma protein binding values of itraconazole at 5 $\mu\text{g}/\text{ml}$ were similar between the control ($97.9\% \pm 0.242\%$) and DMIS ($97.9\% \pm 0.137\%$) rats ($n = 4$ for each). The protein expression of intestinal CYP3A1/2, as determined by Western blot analysis, did not differ between the two groups of rats ($n = 3$ for each) (data not shown). Furthermore, K_m , V_{\max} , and CL_{int} values for the disappearance of itraconazole and the formation of 7-hydroxyitraconazole in both hepatic and intestinal microsomes ($n = 4$) were comparable between the control and DMIS rats (Table 1).

In rats pretreated with dexamethasone, which induces CYP3A1/2, the $AUC_{0-\infty}$ of intravenous itraconazole (20 mg/kg of body weight) was significantly smaller (by 59.4%; 641 ± 110 versus $1,580 \pm 125 \mu\text{g} \cdot \text{min}/\text{ml}$) and the 7-hydroxyitraconazole $AUC_{0-\infty}$ /itraconazole $AUC_{0-\infty}$ ratio was significantly greater (by 113%; $204\% \pm 55.2\%$ versus $95.6\% \pm 12.3\%$) than those in rats without dexamethasone. Conversely, in rats pretreated with troleandomycin, which inhibits CYP3A1/2, the $AUC_{0-\infty}$ of intravenous itraconazole (20 mg/kg) was significantly greater (by 68.2%; $3,380 \pm 873$ versus $2,010 \pm 500 \mu\text{g} \cdot \text{min}/\text{ml}$) and the 7-hydroxyitraconazole $AUC_{0-\infty}$ /itraconazole $AUC_{0-\infty}$ ratio was significantly smaller (by 24.7%; $54.5\% \pm 13.4\%$ versus $72.4\% \pm 9.01\%$) than those in rats without troleandomycin. These data suggest that the metabolism of itraconazole and the formation of 7-hydroxyitraconazole were mediated via hepatic CYP3A1/2 in rats. The amino acid sequences of human CYP3A4 and rat CYP3A1 are 73% identical (8).

The mean arterial plasma concentration-time profiles of itraconazole and 7-hydroxyitraconazole after a 1-min intravenous infusion of 10 mg/kg itraconazole in control and DMIS rats are shown in Fig. 1A and B, respectively. There was no difference in the AUC_{0-24} for itraconazole (516 ± 88.5 and $522 \pm 171 \mu\text{g} \cdot \text{min}/\text{ml}$ in control and DMIS rats, respectively) or 7-hydroxyitraconazole (207 ± 69.6 and $149 \pm 64.8 \mu\text{g} \cdot \text{min}/\text{ml}$, respectively) between the two groups of rats. The demonstration of comparable pharmacokinetics between control and DMIS rats in both the liver and intestines provides major evidence for the efficacy of itraconazole in diabetic patients. This is further supported by comparable hepatic CL_{int} values for the disappearance of itraconazole and the formation of 7-hydroxyitraconazole (Table 1), as the fractions of free itraconazole in the plasma (unbound to plasma proteins) were comparable between the two groups of rats. Itraconazole has a low hepatic extraction ratio in rats (13); the hepatic first-pass effect is almost negligible (11). The above-described data sug-

gest that even if the protein expression and/or mRNA levels of hepatic CYP3A1 (5) and -3A2 (10) were higher in DMIS rats, there was no significant difference in the hepatic metabolism of itraconazole between the control and DMIS rats. The Ae_{0-24} ($<1.02\%$ of the dose) and the GI_{0-24} ($<0.0880\%$ of the dose) for itraconazole were almost negligible. Plasma itraconazole and 7-hydroxyitraconazole were detected only up to 24 h after intravenous administration of itraconazole in the rats (Fig. 1A and B).

The mean arterial plasma concentration-time profiles for itraconazole and 7-hydroxyitraconazole after oral administration of 10 mg/kg itraconazole in control and DMIS rats are shown in Fig. 1C and D, respectively. The AUC_{0-24} for itraconazole (345 ± 94.3 and $308 \pm 154 \mu\text{g} \cdot \text{min}/\text{ml}$ in control and DMIS rats, respectively) and that for 7-hydroxyitraconazole (362 ± 131 and $374 \pm 181 \mu\text{g} \cdot \text{min}/\text{ml}$, respectively) were comparable between the two groups of rats. This may be explained by comparable intestinal CL_{int} rates for the disappearance of itraconazole and the formation of 7-hydroxyitraconazole in both groups of rats, as a result of similar expression levels of intestinal CYP3A1/2 protein. If the present data were to be extrapolated to humans, changes in the dosage regimen of itraconazole would not appear to be required in diabetic patients.

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