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Hydroxy-itraconazole pharmacokinetics is similar to that of itraconazole in immunocompromised patients receiving oral solution of itraconazole

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ABSTRACT

Background: The pharmacokinetic variability of hydroxy-itraconazole (OH-ITZ), an active metabolite of itraconazole (ITZ), is not fully known.
Methods: Oral solution of ITZ was administered in 46 immunocompromised patients as a single 200 mg dose for at least 12 days. The plasma concentrations of ITZ, active OH-ITZ, and keto-itraconazole (keto-ITZ), an inactive metabolite, 12 h after administration were determined by LC–UV or LC–MS/MS.
Results: The mean ± SD of plasma concentrations of ITZ, OH-ITZ, and keto-ITZ were 833 ± 468, 798 ± 454, and 3.94 ± 2.68 μg/l, respectively. A greater correlation coefficient was observed between plasma concentrations of ITZ and OH-ITZ ($r = 0.90$, $P < 0.01$) than between OH-ITZ and keto-ITZ ($r = 0.44$, $P < 0.01$). Plasma concentration of OH-ITZ was inversely correlated with concentration ratio of keto-ITZ to OH-ITZ ($r = -0.52$, $P < 0.01$). Plasma concentrations of ITZ and OH-ITZ were correlated with serum concentration of albumin ($r = 0.36$, $P = 0.01$ and $r = 0.37$, $P = 0.01$) and estimated glomerular filtration rate ($r = -0.27$, $P = 0.08$ and $r = -0.35$, $P = 0.02$).
Conclusions: The pharmacokinetic variability of OH-ITZ was associated with saturated metabolism to keto-ITZ, serum concentration of albumin, and renal function in immunocompromised patients. The plasma concentration of OH-ITZ was strongly correlated with that of ITZ. Prevention of fungal infections can be improved by determining the plasma concentration of ITZ or OH-ITZ.

1. Introduction

Itraconazole (ITZ) is a common triazole antifungal agent prescribed for the treatment and prevention of fungal infections [1]. Monitoring of plasma concentrations of triazole is becoming an important tool with which to minimize its toxicity and ensure efficacy [2]. ITZ is metabolized into more than 30 different metabolites. The main pathway is the formation of hydroxy-itraconazole (OH-ITZ) by CYP3A4 in the liver and/or intestine [3]. OH-ITZ is metabolized to inactive keto-itraconazole (keto-ITZ). Although OH-ITZ was also reported to have antifungal activity in vitro [4], its pharmacokinetics in humans has been studied less than that of ITZ.

The bioavailability of an oral solution of ITZ has been shown to be superior to that of a capsule formulation (55 to 30%) [5,6]. ITZ is very lipophilic and has a large volume of distribution [7], and thus ITZ can be quantified in saliva and the brain [8,9]. The protein binding of ITZ is 97–99% [10,11]. ITZ, OH-ITZ, and keto-ITZ have a triazole ring and inhibit CYP3A [12,13]. Their half-lives can be extended by 26–60% with repeated administration compared to single administration [7]. ITZ is mainly diminished by metabolism and little is found in urine [13]. There is little literature on the urinary excretion of ITZ metabolites. ITZ is also excreted in bile; however, its concentration in bile was reported to be lower than 10 μg/l [14].

Prolonged ITZ therapy has been reported to lead to acquired resistance by *Aspergillus fumigatus* [15]. The dosage individualization of an oral solution of ITZ for prophylaxis of fungal disease has not been established, although the pharmacokinetics of ITZ is variable between patients. The antifungal effect of ITZ is dependent on its plasma concentration and area under the concentration–time curve in patients [3,16–18]. The dependency of the plasma concentration of OH-ITZ has not been adequately studied in clinical settings.

Several reports recommend that the total plasma concentration of ITZ and OH-ITZ should be 750–1000 μg/l for prophylaxis of fungal infections [2,3,16]. The pharmacokinetics of OH-ITZ still needs to be clarified. The aim of this study was to determine the pharmacokinetic

Abbreviations: ITZ, itraconazole; OH-ITZ, hydroxy-itraconazole; Keto-ITZ, keto-itraconazole.

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characteristics of OH-ITZ in immunocompromised patients taking an oral solution of ITZ and to evaluate the usefulness of monitoring OH-ITZ.

2. Material and methods

2.1. Patients and study schedule

The study was performed in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by The Ethics Committee of Hamamatsu University Hospital. The patients received information about the scientific aim of the study, and each patient provided written informed consent.

A total of 46 immunocompromised patients taking an oral solution of ITZ (Itrizole®, Janssen Pharmaceutical K.K, Tokyo, Japan) for prophylaxis of a fungal infection were enrolled. ITZ was administered as a single 200 mg daily dose for at least 12 days. The plasma concentrations of ITZ, OH-ITZ, and keto-ITZ 12 h after ITZ administration were determined using LC–UV or LC–MS/MS. Patient demographics were collected from their medical records. Clinical laboratory values such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), total bilirubin (TB), serum creatinine (Scr), serum albumin (Alb), and C-reactive protein (CRP) were obtained from routine laboratory tests (Table 1). The estimated glomerular filtration rate (eGFR) was calculated using the equation eGFR = [194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ × 0.739 (if female) × body surface area/1.73] [19].

2.2. Sample preparation

ITZ and bifonazole as an internal standard were purchased from LKT Laboratories Inc. (St. Paul, MN) and OH-ITCZ and keto-ITCZ were obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Stock solutions of ITZ (100 mg/l), OH-ITZ (40 mg/l), and keto-ITZ (40 mg/l) were prepared in acetonitrile. Distilled water was produced using an Aquarius® Ultrapure water system (Toyo Seisakusho Kaisha, Ltd., Chiba, Japan). All other reagents were analytical grade and commercially available.

Blood specimens were drawn into tubes containing EDTA. Plasma samples were obtained by centrifugation of the blood samples at 1670 × *g* at 4 °C for 10 min. Two-hundred microliters of plasma was deproteinized with 1000 μl acetonitrile containing 200 ng bifonazole and then centrifuged at 17,900 × *g* at 4 °C for 20 min. The supernatant (approximately 1000 μl) was evaporated to dryness. The residue was reconstituted in 55% acetonitrile containing 5 mmol/l ammonium acetate pH 6.0. The injection volume was 10 μl.

2.3. Determination of plasma concentrations of ITZ and OH-ITZ

Plasma concentrations of ITZ and OH-ITZ were determined using LC–UV. Chromatographic analyses were performed using a Shimadzu Prominence Series HPLC System (Shimadzu Corporation, Kyoto, Japan) equipped with a DGU-20A$_5$R degasser, LC-20AD isopump, SIL-20AC auto sampler, CTO-20AC column oven, SPD-M20A diode array detector, and CBM-20A system controller. Data were collected and analyzed using LabSolutions software, version 5.42SP3 (Shimadzu). Separation was carried out on a TSKgel Super-ODS (2.3 μm, 50 × 4.6 mm I.D., Tosoh) kept at 40 °C with a mixture of 20 mmol/l dipotassium hydrogen phosphate buffer (pH 6.0) and acetonitrile (45:55, v/v). The flow rate was 1.2 ml/min, and the analysis rum was 3 min. The wavelength was set at 260 nm. Chromatographic separation of keto-ITZ was also achieved; however, its lower limit of determination of 100 μg/l, defined as the concentration at which the CV was <20% ($n = 7$), was not sensitive enough. The determination method was validated according to the Guidance of the US Food and Drug Administration [20]. The lower limits of determination of both ITZ and OH-ITZ were 50 μg/l ($n = 7$, CV = 3.06 and 1.47%). Eight calibration standards were prepared in drug-free plasma to test for linearity with the following concentrations: 50, 100, 300, 500, 1000, 2000, 4000, and 8000 μg/l ITZ or OH-ITZ. The correlation coefficients were greater than 0.999 ($n = 8$). Three in-house quality controls, representing the low, medium, and high concentrations, were prepared in drug-free plasma with final concentrations of 300, 1000, and 4000 μg/l ITZ or OH-ITZ. Intra- and interassay accuracies were 95.6–99.2% and 98.0–103.2% for ITZ and 95.8–102.3% and 97.1–103.0% for OH-ITZ. Intra- and interassay CVs were 0.50–2.98% and 0.94–9.94% for ITZ and 0.27–2.35% and 0.80–6.35% for OH-ITZ (Table 2).

Table 1
Patient demographics.

Number of patients [male/female]	46 [20/26]
Age (years)	62 (49–70)
Body weight (kg)	51.9 (46.1–60.9)
AST (IU/l)	24 (16–32)
ALT (IU/l)	34 (21–41)
GGT (IU/l)	43 (28–71)
TB (mg/dl)	0.9 (0.6–1.1)
Alb (g/l)	34 (30–38)
Scr (mg/dl)	0.57 (0.41–0.74)
eGFR (ml/min)	84.0 (63.2–121.5)
CRP (mg/dl)	0.14 (0.04–0.78)

Data are median and interquartile range unless otherwise stated.

Table 2
Parameters of analytical performance of the LC–UV method for ITZ and OH-ITZ.

Theoretical value		Intra-assay (n = 7)			Inter-assay (n = 7)		
		Mean ± SD	Accuracy (%)	CV (%)	Mean ± SD	Accuracy (%)	CV (%)
ITZ (μg/l)	300	298 ± 9	99.2	2.98	310 ± 31	103.2	9.94
	1000	956 ± 27	95.6	2.82	997 ± 46	99.7	4.57
	4000	3898 ± 19	97.4	0.50	3920 ± 37	98.0	0.94
OH-ITZ (μg/l)	300	307 ± 5	102.3	1.50	309 ± 20	103.0	6.35
	1000	958 ± 23	95.8	2.35	971 ± 20	97.1	2.04
	4000	3922 ± 11	98.0	0.27	3923 ± 31	98.1	0.80

2.4. Determination of plasma concentration of keto-ITZ

Plasma concentrations of keto-ITZ were determined using LC–MS/MS on an Agilent 1100 HPLC System (Agilent Technologies, Palo Alto, CA) consisting of a G1312A binary pump, G1367A autosampler, G1379A degasser, and G1316A thermostatted column compartment connected to a TSQ®7000 LC-quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Data were collected and analyzed by Xcalibur™ version 1.2 (Thermo Fisher Scientific). Separations were carried out on TSKgel® ODS-100 V (3 μm, 75 × 2.0 mm I.D., Tosoh) within 7.5 min. The column temperature was 40 °C. The mobile phase was a mixture of 5 mmol/l ammonium acetate (pH 6.0) and acetonitrile (45/55, v/v). The flow rate was 0.3 ml/min. The operating conditions for electrospray ionization were a capillary temperature of 260 °C in the positive ion mode, ionizing energy −30 eV, ion spray voltage 4.5 kV, and sheath gas (N_2) pressure of 15 psi. Keto-ITZ and bifonazole were monitored by the respective transitions of m/z 719.3 to 405.9 and 243.1 to 165.0, respectively. The lower limit of

Table 3
Parameters of analytical performance of the LC–MS/MS method for keto-ITZ.

Theoretical value		Intra-assay (n = 7)			Inter-assay (n = 7)		
		Mean ± SD	Accuracy (%)	CV (%)	Mean ± SD	Accuracy (%)	CV (%)
Keto-ITZ (μg/l)	3	3.2 ± 0.26	106.2	8.28	3.0 ± 0.19	98.7	6.28
	10	10.4 ± 0.19	103.8	0.20	10.3 ± 0.46	102.6	4.53
	40	38.8 ± 1.16	97.1	1.16	39.5 ± 0.87	98.7	2.19