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Intrapulmonary Pharmacokinetics and Pharmacodynamics of Itraconazole and 14-Hydroxyitraconazole at Steady State

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We determined the steady-state intrapulmonary pharmacokinetic and pharmacodynamic parameters of orally administered itraconazole (ITRA), 200 mg every 12 h (twice a day [b.i.d.]), on an empty stomach, for a total of 10 doses, in 26 healthy volunteers. Five subgroups each underwent standardized bronchoscopy and bronchoalveolar lavage (BAL) at 4, 8, 12, 16, and 24 h after administration of the last dose. ITRA and its main metabolite, 14-hydroxyitraconazole (OH-IT), were measured in plasma, BAL fluid, and alveolar cells (AC) using high-pressure liquid chromatography. Half-life and area under the concentration-time curves (AUC) in plasma, epithelial lining fluid (ELF), and AC were derived using noncompartmental analysis. ITRA and OH-IT maximum concentrations of drug (C_{max}) (mean ± standard deviation) in plasma, ELF, and AC were 2.1 ± 0.8 and 3.3 ± 1.0, 0.5 ± 0.7 and 1.0 ± 0.9, and 5.5 ± 2.9 and 6.6 ± 3.1 μg/ml, respectively. The ITRA and OH-IT AUC for plasma, ELF, and AC were 34.4 and 60.2, 7.4 and 18.9, and 101 and 134 μg · hr/ml. The ratio of the C_{max} and the MIC at which 90% of the isolates were inhibited (MIC_{90}), the AUC/MIC_{90} ratio, and the percent dosing interval above MIC_{90} for ITRA and OH-IT concentrations in AC were 1.1 and 3.2, 51 and 67, and 100 and 100%, respectively. Plasma, ELF, and AC concentrations of ITRA and OH-IT declined monoexponentially with half-lives of 23.1 and 37.2, 33.2 and 48.3, and 15.7 and 45.6 h, respectively. An oral dosing regimen of ITRA at 200 mg b.i.d. results in concentrations of ITRA and OH-ITRA in AC that are significantly greater than those in plasma or ELF and intrapulmonary pharmacodynamics that are favorable for the treatment of fungal respiratory infection.

Itraconazole (ITRA) is a triazole antifungal agent that has been used successfully for the treatment of aspergillosis, candidiasis, coccidioidomycosis, blastomycosis, cryptococcosis, and histoplasmosis (7, 10, 13, 25, 32, 35, 39, 40). The recommended oral and intravenous dose in adults is 200 mg once or twice daily. Bioavailability is approximately 55% after administration of the oral solution. At steady state, the maximum concentration of drug in serum (C_{max}), time to C_{max}, minimum concentration of drug in serum (C_{min}), and half-life in humans receiving 200 mg twice daily are approximately 2.3 μg/ml, 4.6 h, 1.9 μg/ml, and 65 h (8, 21, 22, 33). The main metabolite, 14-hydroxyitraconazole (OH-IT), is present in plasma at concentrations that are approximately twice those of the parent drug, and OH-IT is approximately as active, on a weight basis, as ITRA (23, 28). Although ITRA is approved for the treatment of fungal respiratory infection, the in vivo concentrations of ITRA and OH-IT in pulmonary alveolar cells (AC) and pulmonary epithelial lining fluid (ELF) in humans have not been reported. The purpose of this study was to determine the steady-state intrapulmonary pharmacokinetic and pharmacodynamic parameters of orally administered ITRA in healthy volunteers.

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MATERIALS AND METHODS

Study design and subjects. This was a prospective, nonblinded study of the plasma and intrapulmonary concentrations of ITRA at steady state. All subjects gave written informed consent and were required to be 21 to 55 years of age and have a body mass index from 18 to 29 (5). Subjects agreed to abstain from alcohol for 48 h prior to and during the study period. The evaluation included a medical history, physical examination, and baseline laboratory testing, including complete blood count with differential, platelet count, and blood urea nitrogen, serum creatinine, aspartate aminotransferase, alanine aminotransferase, gamma glutaryl transferase, alkaline phosphatase, and total bilirubin urinalysis with microscopy. The evaluation was repeated following bronchoscopy. The serum human chorionic gonadotropin hormone was performed on female subjects at baseline and immediately prior to the first dose. Subjects were excluded if they had a history of clinically significant disease, clinically significant abnormal findings at the screening physical examination (including laboratory tests), intolerance to ITRA, ketoconazole, or lidocaine; if they were pregnant and/or lactating, had a history of drug abuse within 1 year of the start of the study, a positive drug screen, history of smoking within the previous year, or were required to take prescription medications (including oral contraceptives) or nonprescription drugs (including protein pump inhibitors and H-2 antagonists) within 7 days of the study; and if they had known problems with malabsorption or other conditions affecting drug absorption or had received an investigational drug within 30 days prior to the study. Twenty-six subjects were assigned to one of five groups of five subjects, each according to the time of bronchoscopy: 4, 8, 12, 16, and 24 h following the last dose. One extra subject was assigned, in error, to the 8-h group ($n = 6$) but was included in the analysis. The 4-h time period was chosen to approximate the peak (C_{max}) intrapulmonary concentration of ITRA; the 8-h time was chosen as an approximate midpoint between the C_{max} (4 h) and C_{min} (12 h) before the next dose in a 12-h dosing regimen. The 24-h time period was selected to examine the possibility of a long intrapulmonary half-life.

ITRA was administered orally in a dose of 200 mg twice daily for a total of 10 doses on an empty stomach. The first and last (10th) doses of study medication were administered under direct supervision in the General Clinical Research Center at the University of California, San Francisco (UCSF). Subjects were observed for 30 min after the first dose for adverse effects. Doses 2 to 9 were

taken according to verbal and written instructions and documented in a written diary by the subjects. Abnormal laboratory tests that were detected on the repeat evaluation were repeated until normal or near normal.

Bronchoscopy and BAL. Standardized bronchoscopy and bronchoalveolar lavage (BAL) (15–19) were performed in the General Clinical Research Center at 4, 8, 12, 16, or 24 h after the administration of the last dose. Systemic sedation was not used. A fiberoptic bronchoscope (Pentax FB-18BS) was inserted in the right middle lobe. Four 50-ml aliquots of normal saline were instilled and each was immediately aspirated into a trap. The average duration of the bronchoscopy (mean ± standard deviation [SD]) was 3.9 ± 1.2 min. The specimens were kept on ice until they were frozen. The first aspirate was discarded. The second, third, and fourth aspirates were pooled (pooled BAL). The volume of the pooled BAL was measured and recorded. A small aliquot (approximately 3 ml) of the pooled BAL was sent to the clinical laboratory for cell count and differential. A measured volume (30 ml) of the pooled BAL was immediately spun at 400 × *g* for 5 min in a refrigerated centrifuge. The supernatant and the cells were separated and frozen at −70°C until assay. A small aliquot of the supernatant was frozen separately for urea assay.

Blood samples. Blood was obtained for drug assay prior to administration of the first dose and last dose and at the completion of bronchoscopy and BAL.

Specimen handling. Blood samples were kept on ice until centrifugation. The plasma was separated and then frozen at −70°C until assay. Plasma specimens were shipped on dry ice to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center, San Antonio, Texas. BAL and AC drug concentrations were measured in the authors' laboratory at UCSF.

ITRA and OH-IT assay. ITRA and OH-IT in plasma were assayed using a modified and validated reverse-phase high-pressure liquid chromatography method as previously described (42). Calibration curves for standards 0.01 to 5 μg/ml were generated for ITRA and OH-IT by linear regression of the ratio of the peak area of each component to the peak area of the internal standard. The ITRA interday coefficients of variation (CVs) for controls at three concentrations were 5.91, 6.06, and 4.98%. For OH-IT, the CVs were 7.60, 8.94, and 8.21%.

BAL fluid and AC specimens were analyzed in our research laboratory. The cell pellet was reconstituted in water to a 10-fold concentration of the BAL fluid volume that produced the pellet, and then it was sonicated for 2 min with a model 550 Sonic Dismembrator (Fisher Scientific, Santa Clara, Calif.). Specimens were stored at −80°C until assayed.

ITRA and OH-IT in BAL fluid and AC were measured by a modified and validated reverse-phase high-pressure liquid chromatography method as previously reported (14). ITRA, OH-IT, and the internal standard were supplied by Janssen Pharmaceutica (Beerse, Belgium). Standard curves were constructed by weighted (reciprocal of the concentration) least-squares linear regression of the peak height ratios of ITRA or OH-IT to the internal standard versus the nominal concentration. The lower limit of quantitation was 2.75 ng/ml for ITRA and 2.5 ng/ml for OH-IT. The correlation coefficients (*r*) were at least 0.99 for both compounds.

Interday and intraday validation was performed at three concentrations: low, medium, and high. The accuracy ranges for ITRA, defined by the relative error, for all interday and intraday assays together were −13.5 to 21.9% and −14.0 to 15.9% for BAL and leukopak, respectively. For OH-IT, the ranges of relative error were −19.4 to 9.2% and −15.7 to 12.9%. The precision of the assay, defined by the mean (± SD) CV, was 6.5% ± 3.5% and 4.5% ± 2.0% for ITRA and 4.0% ± 1.2% and 4.5% ± 3.2% for OH-IT in BAL and leukopak, respectively. Stability studies of controls stored frozen at −80°C for 6 weeks showed no degradation of the drug or its metabolite.

Quantitation of volume of ELF and concentration of antibiotics in ELF and AC. The amount of ELF recovered was calculated by the urea dilution method (34), as reported in previous pulmonary pharmacokinetic studies (15–19). The concentration of urea in serum was analyzed by the clinical laboratory at UCSF using a coupled urease-glutamate dehydrogenase enzymatic method modified by Boehringer Mannheim Corporation (Indianapolis, Ind.) (38). Urea was measured in BAL supernatant by utilizing a modified enzymatic assay (blood urea nitrogen kit UV-66; Sigma, St. Louis, Mo.), as previously reported (15–19). The assay was linear ($R^2 = 0.99$) for concentrations of urea in BAL fluid from 0.047 to 0.750 mg/dl. Controls were included with every run, and if they were not within 10% of the known value, the standard curve, controls, and specimen assays were repeated.

The volume of ELF in bronchoalveolar lavage fluid, the concentration of antibiotic in the ELF, and the concentration of antibiotic in alveolar cells were derived using methods and calculations that have previously been published (11–15). The volume of AC collected in the pellet suspension was determined from the cell count in the BAL fluid. Because of cell loss during centrifugation, the actual number of cells recovered may be lower than the number counted, and the antibiotic concentration may be approximately 20% greater than we calculated (41). Differential cell counting was performed after spinning the specimen in a cytocentrifuge. The volume of alveolar cells in the pellet suspension was determined using a mean macrophage cell volume of 2.42 μl/10^6 cells (6).

Statistical, pharmacokinetic, and pharmacodynamic analysis. Descriptive statistics, graphic representations, and database management were performed using PROPHET, version 6.0 (Division of Research Resources, National Institutes of Health, Bethesda, Md., and MarketMiner Inc., Charlottesville, Va.). Noncompartmental modeling was performed using Kinetica 2000, version 4.0.1 (InnaPhase Corporation, Philadelphia, Pa.). Because the interpatient variability of plasma, ELF, and AC ITRA concentrations, at each of the selected time periods, was not known prior to the study, we used sample sizes (five in each group) based upon our prior experience with linezolid and rifapentine and a similar study design (17, 19). We estimated that at the 8-h time period (C_{max}) and with similar interpatient variability, we would be able to detect an approximate 65% difference between the means of the plasma and ELF or AC concentrations with a power of 80% and α of 0.05. For lesser or greater degrees of interpatient variability, the differences that we would be able to detect would be smaller or larger, respectively. The log-trapezoidal rule was used to compute the area under the curve (AUC) from 0 to 24 h for the mean concentration-time data in plasma, ELF, and AC after the 10th dose. The plasma, intracellular, and ELF concentration-time data declined monoexponentially; the means of the observed concentrations at each BAL time were used to calculate L_Z, the elimination rate constant. Fitting was performed using a weighting function, $1/Y^2$, where $1/Y$ was the reciprocal of the observed concentration. The plasma, ELF, and AC half-lives were calculated using the following relationship: $t_{1/2} = 0.693/L_Z$.

Analysis of variance was used to compare the concentrations in plasma, AC, and ELF and ELF recovery and AC recovery at the different time periods. Prior to performing the analysis of variance, the data sets were tested for normality (Wilk-Shapiro) and equality of variances (Levene's test). Parametric and nonparametric analyses were performed using the Newman-Keuls or Friedman tests, respectively (43). The AC/plasma and ELF/plasma ratios were calculated by averaging the ratios for the five subjects at each collection time point. Linear regression was performed using the method of least-squares estimation. A P of <0.05 was regarded as significant. The MICs at which 90% of the isolates were inhibited (MIC_{90}s) of ITRA used for the pharmacodynamic calculations were obtained from recent publications: *Candida albicans* (MIC_{90} = 0.12 μg/ml) (30), *Coccidioides immitis* (MIC_{90} = 0.25 μg/ml) (20), *Aspergillus flavus* (MIC_{90} = 1 μg/ml) (31), and *Aspergillus fumigatus* (MIC_{90} = 2 μg/ml) (31). The MIC_{90}s of OH-IT for these organisms are not known; however, the activity of ITRA and OH-IT were reported to be essentially similar for 1,481 isolates of pathogenic fungi (28). Thus, the pharmacodynamic calculations were performed using the same MIC_{90}s for ITRA and OH-IT.

RESULTS

Twenty-six subjects were enrolled in the study. An extra patient was enrolled by error into the 8-h time group and was included in the analysis. Thirty-one subjects were consented; 26 completed the study, 3 subjects dropped out after signing the consent form and prior to screening, 1 subject developed a rash on the chest and arms after the second dose and was discontinued, and 1 subject failed to report for the last dose and bronchoscopy. The ages of the 26 enrolled subjects ranged from 23 to 50 with a mean (± SD) of 34 ± 8 years. All had normal renal function with serum creatinines that ranged from 0.7 to 1.2 mg/dl. The remaining screening laboratory tests were within normal limits. There were no major adverse events, and the subjects returned to their normal duties following the bronchoscopy and BAL. None experienced chest pain or shortness of breath. Two subjects developed transient fever and two others experienced self-limited postbronchoscopy cough. Transient lightheadedness occurred in 7 and rales and/or diminished breath sounds were present in 18 of the 26 subjects following the bronchoscopy. On repeat laboratory testing, four subjects had elevated serum transaminases, two had an elevated serum bilirubin, three subjects had a mildly decreased

TABLE 1. Recovery of cells and ELF from BAL fluid[a]

Parameter[b]	Value for group (h)				
	4	8	12	16	24
No. of subjects	5	6	5	5	5
Mean (cells/liter)	9.9×10^7	9.5×10^7	9.5×10^7	11.5×10^7	12.6×10^7
SD (cells/liter)	5.5×10^7	3.5×10^7	3.3×10^7	4.4×10^7	2.3×10^7
PMNs (%)	2.4 ± 1.8	1.2 ± 1.0	2.8 ± 3.1	1.8 ± 1.5	1.4 ± 1.1
Lymphocytes (%)	14.4 ± 3.7	14.7 ± 12.0	8.4 ± 7.4	16.6 ± 11.9	16.0 ± 6.9
Mono/macro (%)	82.6 ± 3.8	80.2 ± 12.7	88.2 ± 8.2	81.6 ± 12.2	76.6 ± 17.2
Eosinophils (%)	0.6 ± 0.9	0.8 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4
Other cells (%)	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.9
Degen cells (%)	0.0 ± 0.0	2.8 ± 4.3	0.6 ± 1.3	0.0 ± 0.0	5.4 ± 12.1
ELF volume (ml)	1.2 ± 0.9	0.9 ± 0.3	1.1 ± 0.7	0.9 ± 0.4	1.0 ± 0.3

[a] All data given as means ± SDs. There were no significant differences among the groups for cell recovery, differential cell count, or volume of ELF ($P > 0.05$). Comparison testing was not applied to zero values.
[b] PMN, polymorphonuclear leukocyte; Mono/macro, monocytes and macrophages; Other, other unrecognizable cells; Degen, degenerated cells.

hemoglobin or hematocrit measurement, two had a trace of protein, and three had trace blood in the urine, all of which returned to normal or toward normal.

The number (mean ± SD) of AC recovered from BAL fluid ranged from $9.5 \times 10^7 \pm 3.5 \times 10^7$ to $12.6 \times 10^7 \pm 2.3 \times 10^7$ (Table 1). Alveolar cell recovery was not significantly different among the five time groups ($P > 0.05$). The majority of the cells in all time groups were in the monocyte/macrophage class (range 76.6% ± 17.2% to 88.2% ± 8.2%). The volume (mean ± SD) of ELF recovered from the 26 subjects was 1.0 ± 0.5 ml and was not significantly different among the time groups ($P > 0.05$) (Table 1). The plasma, AC, and ELF ITRA and OH-IT concentrations are summarized in Table 2.

There was a weak but significant negative correlation ($R = -0.47$; $P = 0.01$) between the weights of the subjects and the concentrations of ITRA in plasma at 12 h following the ninth dose. There was a stronger negative correlation ($R = -0.63$; $P = 0.0005$) between the weights of the subjects and the concentrations of OH-IT in plasma at 12 h following the ninth dose. The OH-IT/ITRA concentration ratio in plasma at 12 h following the ninth dose was 2.2 ± 0.5. For all time periods, the ELF OH-IT/ITRA concentration ratio was 2.2 ± 0.9 (P of >0.05 compared with plasma). The AC OH-ITRA concentration ratio was 1.6 ± 0.6 (P of <0.05 compared with plasma and ELF), indicating greater metabolite concentrations in plasma and ELF than in AC. The clinical significance of the differences observed in the OH-IT/ITRA ratios is unknown.

The mean (± SD) plasma ITRA concentrations determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h after the 10th dose) ranged from 2.1 ± 0.8 μg/ml at 4 h (plasma C_{max}) to 0.9 ± 0.4 μg/ml (plasma C_{min}) at 24 h and declined monoexponentially with a half-life of 23.1 h. The plasma AUC (0 to 24 h) for ITRA was 34.4 μg · h/ml. The mean (± SD) plasma OH-IT concentrations determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h after the 10th dose) ranged from 3.3 ± 1.0 μg/ml (plasma C_{max}) at 4 h to 2.0 ± 0.5 μg/ml (plasma C_{min}) at 16 h and declined monoexponentially with a half-life of 37.2 h. The plasma AUC (0 to 24 h) for OH-IT was 60.2 μg · h/ml.

The mean (± SD) ELF concentrations of ITRA determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h following the 10th dose) ranged from 0.5 ± 0.7 μg/ml at 12 h (ELF C_{max}) to 0.2 ± 0.1 μg/ml at 24 h (ELF C_{min}). The ELF AUC (0 to 24 h) for ITRA was 7.4 μg · h/ml. The mean (± SD) ELF concentrations of OH-IT determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h following the 10th dose) ranged from 1.0 ± 0.9 μg/ml at 12 h (ELF C_{max}) to 0.6 ± 0.2 μg/ml at 24 h (ELF C_{min}). The ELF AUC (0 to 24 h) for OH-IT was 18.9 μg · h/ml. The mean ELF concentrations of ITRA and OH-IT declined monoexponentially with half-lives of 33.2 and 48.3 h, respectively.

The mean (± SD) AC concentrations of ITRA determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h following the 10th dose) ranged from 5.5 ± 2.9 μg/ml (AC C_{max}) at 4 h to 2.1 ± 1.0 (AC C_{min}) at 24 h. The AC AUC (0 to 24 h) for ITRA was 101 μg · h/ml. The mean (± SD) AC concentrations of OH-IT determined at the time of bronchoscopy (4, 8, 12, 16, and 24 h following the 10th dose) ranged from 6.6 ± 3.1 μg/ml

TABLE 2. ITRA and OH-IT concentrations in plasma, ELF, and AC[a]

BAL group (h)	Plasma concn 12 h after the ninth dose (μg/ml)[b]		Plasma concn at the indicated BAL time (μg/ml)[c]		AC concn at the indicated BAL time (μg/ml)[c]		ELF concn at the indicated BAL time (μg/ml)[c]	
	ITRA	OH-IT	ITRA	OH-IT	ITRA	OH-IT	ITRA	OH-IT
4	1.1 ± 0.4	2.7 ± 1.0	2.1 ± 0.8	3.3 ± 1.0	5.5 ± 2.9	6.3 ± 2.8	0.3 ± 0.3	0.8 ± 0.5
8	1.1 ± 0.5	2.0 ± 0.6	1.2 ± 0.3	2.5 ± 0.5	4.0 ± 1.4	4.9 ± 1.7	0.3 ± 0.3	0.8 ± 0.3
12	0.8 ± 0.1	1.7 ± 0.4	0.9 ± 0.3	2.0 ± 0.7	4.9 ± 2.0	6.6 ± 3.1	0.5 ± 0.7	1.0 ± 0.9
16	1.1 ± 0.4	1.9 ± 0.6	1.2 ± 0.4	2.0 ± 0.5	3.7 ± 2.7	5.4 ± 2.1	0.3 ± 0.3	0.8 ± 0.4
24	1.0 ± 0.5	2.2 ± 1.0	0.9 ± 0.4	2.2 ± 0.9	2.1 ± 1.0	4.3 ± 0.9	0.2 ± 0.1	0.6 ± 0.2

[a] Data are given as means ± 1 SD. OH-IT concentrations were greater than ITRA concentrations in all cases ($P < 0.05$).
[b] There were no significant differences among the plasma concentrations of ITRA or OH-IT at 12 h after the ninth dose ($P > 0.05$).
[c] AC > plasma > ELF ITRA concentrations ($P < 0.05$), AC > plasma > ELF OH-IT concentration ($P < 0.05$).

(AC C_{max}) at 12 h to 4.3 ± 0.9 (AC C_{min}) at 24 h. The AC AUC (0 to 24 h) for OH-IT was 134 μg · h/ml. The mean AC concentrations of ITRA and OH-IT also declined monoexponentially with half-lives of 15.7 and 45.6 h.

DISCUSSION

In the present study, ITRA and OH-IT concentrations were significantly greater in AC than in plasma, both of which were greater than the concentrations in ELF, indicating enhanced cellular penetration and relative exclusion from the ELF compartment. The observations are consistent with those of Perfect et al. (29), who demonstrated uptake of ITRA by alveolar macrophages and an uptake ratio (intracellular concentration/extracellular concentration) of 70 in serum free medium, 18 in 5% serum, and 3 in 100% serum. The latter value is comparable to the overall AC/plasma ITRA ratio of 3.4 ± 1.9 observed in this study. The physiological basis for this differential penetration is unknown but the lipophilic, hydrophobic nature of ITRA favors its entry into cells from an aqueous environment (29). This and the high protein binding of itraconazole (>99%) would also explain, in part, why ITRA is present in significantly lower concentrations in ELF.

AUC/MIC ratios of ≥100 and percent time above MIC of ≥85% in plasma have been associated with optimal efficacy for antibiotic treatment of gram-positive infections in humans (26). Less is known regarding the correlation between antifungal pharmacodynamics and clinical outcomes in humans. In in vitro studies using time-kill curves, ITRA was most effective when drug concentrations were maintained at 8 to 16 times the MIC of *Cryptococcus neoformans* (11) and 2 times the MIC of *Candida albicans* (12). In another in vitro study utilizing time-kill curves, the fungicidal activity of ITRA against *A. fumigatus* conidia was concentration dependent between 1.25 and 10 μg/ml (27). Inhibitory but not fungicidal activity was demonstrated against *Candida albicans* at the same concentrations. Additional in vitro studies have demonstrated that the AUC/MIC ratio and the time above MIC are important parameters in assessing the antifungal activity of azoles (36, 37, 44). In a neutropenic, murine model of disseminated candidiasis, the AUC/MIC ratio was predictive of efficacy for fluconazole, posaconazole, ravuconazole, and voriconazole (1–4). In a meta-analysis of 38 clinical trials involving 7,014 patients, greater azole dose, among other factors, was identified as a predictor of a positive treatment effect (9). These data indicate that the antifungal efficacy of itraconazole and other azoles is concentration-time dependent.

In the present study, the calculated plasma C_{max}/MIC_{90} ratios for *A. fumigatus*, *A. flavus*, *Coccidioides immitis*, and *Candida albicans* were 1.1, 2.1, 8.4, and 17.5 for ITRA and 1.7, 3.3, 13.2, and 27.5 for OH-IT. The AUC/MIC_{90} ratios were 17.2, 34.4, 138, and 287 for ITRA and 30.1, 60.2, 241, and 502 for OH-IT. ITRA and OH-IT concentrations were above the MIC_{90}s for *Candida albicans*, *Coccidioides immitis*, and *A. flavus* for 75 and 100% of the dosing interval. For *A. fumigatus*, the plasma concentrations of ITRA and OH-IT were above the MIC_{90} for 33 and 100% of the dosing interval. Plasma pharmacodynamics of ITRA or OH-IT have not been correlated with clinical outcomes in humans.

The calculated ELF C_{max}/MIC_{90} ratios for *A. fumigatus*, *A. flavus*, *Coccidioides immitis*, and *Candida albicans* were 0.2, 0.3, 1.3, and 2.5 for ITRA and 0.3, 0.6, 2.4, and 5.0 for OH-IT. The AUC/MIC_{90} ratios were 3.7, 7.4, 29.6, and 61.7 for ITRA and 9.5, 18.9, 75.6, and 158 for OH-IT. ITRA and OH-IT concentrations were above the MIC_{90}s for *Candida albicans* for 100% of the dosing interval and for 80 and 100% of the dosing interval for *Coccidioides immitis*. For *A. flavus* and *A. fumigatus*, the ELF concentrations of ITRA or OH-IT were not above the MIC_{90} for any portion of the dosing interval. The role of ELF ITRA concentrations in the treatment of *Aspergillus* or other fungal pulmonary infection is unknown, but these data suggest that they may not be important since patients respond to therapy despite these relatively low ratios.

The calculated AC C_{max}/MIC_{90} ratios for *A. fumigatus*, *A. flavus*, *Coccidioides immitis*, and *Candida albicans* were 1.1, 5.5, 22, and 45.8 for ITRA and 3.2, 6.3, 25.2, and 52.5 for OH-IT. The AUC/MIC_{90} ratios were 51, 101, 404, and 842 for ITRA and 67, 134, 536, and 1117 for OH-ITRA. The percent time above the MIC_{90} for ITRA and OH-IT in ELF was 100% of the 12-h dosing interval for *Candida albicans*, *Coccidioides immitis*, *A. flavus*, and *A. fumigatus*.

Fluconazole is 11 to 12% protein bound in many species (24). More recently developed azoles, e.g., itraconazole (99%), voriconazole (78%), posaconazole (99%), and ravuconazole (96%) are highly protein-bound molecules (36, 37, 44). Zhanel et al. concluded that protein binding resulted in reduction in antifungal activity, either related to alteration in MIC, fungicidal activity, or postantifungal effect (44). We did not measure protein binding in this study or its effect on the concentrations of itraconazole or the calculated pharmacodynamics. Our assay measured total (free and protein-bound) ITRA or OH-IT concentrations in plasma, ELF, and AC; thus, the fraction of free drug was not determined. It is likely that free drug concentrations and the pharmacodynamic ratios were less than those that we have reported and that this effect would be greatest in the serum. Further investigation is warranted to determine the effect of protein binding on the pharmacodynamics of itraconazole in these compartments.

It is likely that intracellular antifungal activity is important in the clinical response to therapy (29). However, the relative importance of plasma, ELF, and AC itraconazole concentrations cannot be determined from our study. Our data indicate that the oral 200 mg twice-daily dosing regimen, used in normal subjects in this study, yields high alveolar cell drug AUC/MIC ratios and concentrations that continuously exceed the MIC_{90}s of target pathogens. It is not known whether similar concentrations are achieved in sick patients. However, this observation is consistent with the reported success of therapy with itraconazole for fungal respiratory infection (21). Since the bioavailability of oral itraconazole is 55%, we would expect that intravenous itraconazole administered prior to oral therapy would alter these pharmacokinetic and pharmacodynamic observations. The relationship of these pharmacodynamic parameters in plasma, ELF, and AC to clinical outcomes merits further investigation.

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