Pharmacokinetic evaluation of oral itraconazole for antifungal prophylaxis in children

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Article type : Rapid Communication

TITLE PAGE

Pharmacokinetic evaluation of oral itraconazole for antifungal prophylaxis in children.

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Short title: ITC TDM in paediatrics.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1440-1681.12822

* PHASE I AIFA, UNI EN ISO 9001:2008 and 13485:2012 (CE-IVD) CERTIFIED LABORATORY; Certificate No. IT-64386; ** Certification for: ”DESIGN, DEVELOPMENT AND APPLICATION OF DETERMINATION METHODS FOR ANTI-INFECTIVE DRUGS. PHARMACOGENETIC ANALYSES.”

ABSTRACT

Itraconazole is a first-generation triazole agent with an extended spectrum of activity; it is licensed in adults for superficial and systemic fungal infections; no recommendation has been yet established for use in children patients. Its variable and unpredictable oral bioavailability make it difficult to determine the optimal dosing regimen. Hence, therapeutic drug monitoring, highly available in clinical practice, may improve itraconazole treatment success and safety. The aim of the study was to describe in paediatrics the oral itraconazole pharmacokinetics, used for prophylaxis. Moreover, we evaluated the utility of its therapeutic drug monitoring in this cohort. A fully validated chromatographic method was used to quantify itraconazole concentration in plasma collected from paediatric patients, at the end of dosing interval. Associations between variables were tested using the Pearson test. Mann-Whitney U test has been used to probe the influence of categorical variables on continuous ones. Any predictive power of the considered variables was finally evaluated through univariate and multivariate linear and logistic regression analyses. A high inter-individual variability was shown; ethnicity (beta coefficient, β: -0.161 and interval of confidence at 95%, IC: -395.035;-62.383) and gender (β: 0.123 and IC: 9.590; 349.395) remained in the final linear regression model with *p value* of 0.007 and 0.038, respectively. This study highlights that therapeutic drug monitoring is required to achieved an adequate target itraconazole serum exposure.

KEYWORDS: therapeutic drug monitoring; itraconazole; HPLC; antifungal; invasive fungal infections; paediatrics.

1. INTRODUCTION

Invasive fungal infections (IFIs) are a significant cause of morbidity and mortality in children. Successful management of these systemic infections requires identification of the causative pathogen, appropriate antifungal selection, and optimisation of its pharmacokinetic and pharmacodynamic properties, to maximise its antifungal activity and minimise toxicity and the emergence of resistance (1). Azoles remain the first choice for prevention and treatment of IFIs(2); however, the clinical use of these drugs is characterized by frequent pharmacological disadvantage in terms of pharmacokinetic variability and drug-drug interactions (3).

Itraconazole (ITC, Sporanox®) is a first-generation azole approved by the United States Food and Drug Administration (FDA) for the treatment of fungal infections; it is well tolerated and effective for superficial and systemic fungal infections; no recommendation has been yet established for use in children patients (4). ITC is lipophilic and it interferes with the synthesis of the cytochrome P450 (CYP)-dependent enzyme lanosterold 14-α-emethylase, an ergosterol precursor essential for the fungal cell membranes (5, 6). ITC levels are 2 to 20 times higher in tissues, such as lungs, kidney, bone and muscles, skin, nails, and the female genital tract, than in the serum; nevertheless penetration into the cerebrospinal fluid is limited (7). It is metabolized in liver, yielding over 30 different metabolites. In pediatric patients, ITC absorption rate from oral (OS) administration was found to be greater than capsules and higher drug plasma exposure has been observed in older children (5 to 12 years old) (8-12).

Drug levels and treatment outcome depend on host factors, target organisms and associated interventions and therapeutic drug monitoring (TDM) can timely and appropriately guide drug dosage modifications (13). Already published clinical TDM studies have been conducted and they observed that ITC dose modification could result in more appropriate drug levels (14). The aim of our study was to describe, in paediatrics, the ITC OS pharmacokinetics, used for IFIs prophylaxis (OS administration), and to evaluate the utility of ITC TDM in this population.

2. RESULTS

Mean, standard deviation (SD), median and median and interquartile range (IQR), 25th to 75th percentiles, values for age, body mass index (BMI) and ITC plasma concentrations were resumed and compared in table 1; there were no statistically significant differences in terms of baseline characteristics.

The drug dosage has been evaluated with a score from 1 to 18, as showed in Table 2.

Based on published ITC through levels cut-off for prophylaxis (9-11), we observed that 128 patients (44.1%; median 78.00 ng/mL, IQR: <limit of detection (LOD) - 148.00 ng/mL) showed sub-optimal exposure, whereas 162 subjects (55.9%; median 735.00 ng/mL; IQR: 431.50 - 1203.75 ng/mL) had concentrations higher than the efficacy defined level.

A high interindividual variability was found between ITC C_{trough} concentrations: the median value was 306.50 ng/mL and the IQR range was 91.00 ng/mL and 781.25 ng/mL.

Pearson test showed that there were no statistically significant correlation among ITC C_{trough} and drug dose, BMI or age. Mann-Whitney U did not result in statistically significant influence of sex or ethnicity on ITC exposure.

Univariate linear regression analysis was performed to evaluate the effect of ethnicity, gender, age, BMI and drug dose on ITC C_{trough}. Stepwise forward regression analysis was used to identify the minimum set of independent predictive variables of ITC exposure and estimate the contribution of each factor to pharmacokinetic variability; only ethnicity (beta coefficient, β: -0.161 and interval of confidence at 95%, IC: -395.035;-62.383) and gender (β: 0.123 and IC: 9.590; 349.395) remained in the final model with *p* value of 0.007 and 0.038, respectively (Table 3).

Univariate logistic regression analysis was carry out to evaluate the effect of age, gender, BMI and drug dose on ITC efficacy cut-off value of 250 ng/mL. Stepwise forward regression analysis was used

to identify the minimum set of independent predictive variables of the cut-off effectiveness and estimate the contribution of each factor to pharmacokinetic variability; no factors remained in the final model (table 4).

3. DISCUSSION

ITC pharmacokinetics are highly variable, probably due to its unpredictable oral bioavailability (15, 16): it is non-linear (or saturable) exhibits prolonged clearance and slow accumulation (17). The drug half-life is approximately 24 h and the time to steady state is about 14 days (16). ITC is a weakly basic Biopharmaceutics Classification System (BCS)(18) class II (low solubility/high permeability) drug with a pH-dependent dissolution (pKa value, 3.7); for this reason it requires an acidic gastric environment for adequate dissolution and absorption. In fact, ITC coadministration with gastric acidity inhibitors, such as antacids and proton pump inhibitors, reduce the extent of drug absorption (19, 20). ITC is a substrate of P-glycoprotein (P-gp), a membrane efflux transporter (21-24).

Our results show that ITC exposure has a high interindividual variability and participants age, BMI, ethnicity, sex or age did not significantly influence ITC pharmacokinetics. Instead, linear regression analysis showed that ethnicity (p=0.007) and gender (p=0.038), respectively are negative and positive, predictors of trough levels (Table 3). Gender-related differences, such as body size and muscle mass, may result in pharmacokinetic differences; genetic variations among ethnic groups also can alter drug disposition (25).

Ethnicity may influence the disposition of P-gp-substrate drugs due to the of differences in P-gp polymorphism between black and white subjects. Especially, P-gp polymorphisms may influence ITC:

in a study on ITC, administered before fexofenadine, a P-gp substrate, the fexofenadine area under the concentration curve was significantly higher and the drug clearance significantly lower in individuals with TT phenotype than in GC haplotype, indicating P-gp inhibition by ITC in TT subjects (26).

During the past decade many information about inter-gender differences has been published in adult population (25, 27-30). Considering the pharmacokinetic differences, an apparent high female CYP3A4 activity has been reported (31). Conversely, the activity of other CYP isozymes (e. g. CYP2C19, CYP2D6, and CYP2E1) and the glucuronidation activity may be higher in males (28, 29). Thus, different oral bioavailability, caused by different intestinal and hepatic metabolic enzymes activity, may be found. However, no evidence about children are still available.

Moreover, women have lower acid secretion in the stomach (28), and then the ITC absorption might be compromised, due to its incomplete dissolution and unrestricted presystemic intestinal metabolism. Eventually, in a study on 639 cases of invasive candidiasis, non-white race and female gender were more commonly associated with non-albicans species (32).

Evaluating the logistic regression analysis, performed to assess the effect of age, gender, BMI and drug dose on ITC efficacy cut-off for prophylactic use, no factors were retained in the final model (table 4). We chose to consider the steady-state itraconazole trough concentration of 250 ng/mL (9-11) for our analyses, because it is the one used by our clinicians to discriminate prophylaxis outcome. Nevertheless, more recent publication describe a new threshold for prophylaxis of 500 ng/mL (14, 33, 34).

To our knowledge, in literature there are limited data describing the ITC use in children; ITC single intravenous or OS dose of 2.5 mg/kg/day in children aged 7 months to 17 years is well tolerated, but it results in a high variability in drug exposure (35). Moreover, OS dose of 5 mg/kg/day results in lower concentrations in infants compared with children older than 2 years of age (36).

ITC remains a key agent for the management of endemic mycoses worldwide and its broad spectrum of activity and availability (intravenous and OS route of administration) suggest that ITC long-term use is affordable and practical. In addition, the ITC pharmacokinetic variability its numerous potential drug interactions prompt that TDM is clinically necessary in order to achieve safe and effective systemic drug exposures. The results from the present study might be further explained through pharmacogenetic analyses, which could explain posaconazole levels variability, also concerning gender, and why it is ineffective in some patients (37).

Potential limitations to our study include its retrospective nature and a relatively small sample size; moreover it lacks of a standardized protocol for ITC dosing. Hence, further works applied to larger cohorts and which include the ITC active metabolite (hydroxyitraconazole) quantification are required to confirm the reported data.

4. METHODS

2.1 Patients and inclusion criteria

Plasma samples were collected at the Laboratory of Clinical Pharmacology and Pharmacogenetics (Department of Medical Sciences, Unit of Infectious Diseases, University of Turin, Amedeo di Savoia Hospital, Turin) and Clinical Pharmacology Service "Franco Ghezzo" (Department of Biological and Clinical Sciences, University of Turin, S. Luigi Gonzaga Hospital) from different Hospitals in Piedmont (Italy). Inclusion criteria were: age below 18 years old, diagnosed IFI and treatment with oral ITC for prophylaxis, with an adherence of 90%. Patients on treatment with potential interacting drugs, allergy or intolerance to ITC, HIV infection, severe malnutrition, liver cirrhosis, chronic renal failure (with estimated creatinine clearance, eCRCl<60 mL/min) were excluded. Two hundred ninety paediatric patients (175 males, 60.3 %), treated with ITC, were enrolled. About half of them (51.7%; N=150) were Caucasians. All the enrolled patients received ITC antifungal prophylaxis and the route of administration was only OS.

The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional local Ethics Committee (study protocol: "PkPG_J02AC Studio retrospettivo per la valutazione e farmacocinetica e farmaco-genetica della terapia antimicotica con farmaci triazolici"). A written informed consent for the study was obtained from each subject, signed by natural/biological father or mother of a child with full parental legal rights. The primary aim of the approved protocol consist in: evaluation of triazoles plasma trough concentration at the steady state condition, and correlation of the obtained data with treatment outcome and toxicity.

For all the patients, following data were available: gender, age, BMI, ethnicity and ITC dose.

2.2 Determinations of ITC plasma concentration

Patient blood samples (collected in lithium-heparin tube, 5 mL) were taken immediately before drug intake (C_{trough}), under steady-state conditions (reached after two weeks of ITC oral solution). Plasma samples were obtained by centrifugation at 3000 rpm for 10 min at 4°C. 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline (QX), used as the internal standard (IS), was purchased from Sigma-Alderich Corporation (Milan, Italy), and ITC was purchased from Sigma-Alderich Corporation (Milan, Italy). Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from VWR (Milan, Italy). Formic acid was from Sigma-Alderich Corporation (Milan, Italy). HPLC-grade water was produced by a Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy).

Two hundred µl of plasma samples were pipetted into a polytetrafluoroethylene tube, and 50 µl of IS working solution was added to each tube. Samples were extracted by protein precipitation using 200 µl of acetonitrile. Each sample was vortexed for at least 15s and then centrifuged at 12,000 rpm for 10 min at 4°C. One hundred µl of supernatant was transferred to a glass vial and diluted with 100 µl of water. Fifty µl of sample was then injected into the HPLC-MS system. All extraction procedures were performed at room temperature. The HPLC-MS system used was a Waters system (Milford,

MA), with a binary pump (model 1525), in-line degasser AF, 717-plus autosampler, and Micromass ZQ mass detector. The LC-MS Empower 2 Pro software program (version year 2005; Waters) was used (38, 39).

Chromatographic separation was performed at 35°C, using a column oven, on a C18 Atlantis T-3 5-µm (150 mm by 4.6 mm, inside diameter [i.d.]) column (Waters, Milford, MA), protected by a Security Guard with a C18 (4.0 mm by 3.0 mm, i.d.) precolumn (Phenomenex; CA). The mobile phase composed initially of 50:50 water with formic acid (0.05%)/acetonitrile with formic acid (0.05%) was then ramped to 20:80 within 6.5 min. The flow rate was set at 1 mL/min.

Detector settings were as follows: electrospray ionization (ESI^+); capillary voltage, 3.5 kV; source temperature, 110°C; desolvation temperature, 350°C; nitrogen desolvation flow, 400 L/h; nitrogen cone flow, 50 L/h. The ion *m/z* values monitored were: 353.2 for ITC and 313.4 for QX, cone voltage was 25 V and 50, respectively.

The lower limit of quantification (LLOQ) was considered the lowest standard on the calibration curve. Therefore, the LLOQ for ITC was 0.031 µg/ml. The considered LOD was 0.015 µg/ml. Intra- and interday precision were calculated by determining the relative standard deviation (% RSD) at each QC concentration, as shown in Table 5.

This work was carried out in a PHASE I AIFA, UNI EN ISO 9001:2008 and 13485:2012 (CE-IVD) certified laboratory.

2.3 Statistical Analysis

For descriptive statistics, continuous and non normal variables were summarized as average, SD, IQR 25th to 75th percentiles was calculated to measure the statistical dispersion of the data; categorical variables were described as frequency and percentage. All the variables were tested for normality with the Shapiro-Wilk test. The correspondence of each parameter was evaluated with a normal or non-normal distribution, through the Kolmogorov-Smirnov test.

The Independent Samples *t* Test was used to compare the means of two independent groups, considering the level of statistical significance (*p* value<0.05). Pearson linear correlation coefficient (r) was used to investigate the strength of the association between two quantitative variables considering the level of statistical significance (*p* value<0.05). Mann-Whitney U test was used to probe the influence of categorical variables on continuous ones, considering the level of statistical significance (*p* value<0.05). Any predictive power of the considered variables was finally evaluated through univariate and multivariate linear (for pharmacokinetic parameters) and logistic (considering prophylaxis efficacy cut-off (9-11)) regression analyses. Factors with a *p value* <0.2 in univariate analysis were considered in multivariate analysis (*p value* <0.05).

All the tests were performed with IBM SPSS Statistics 22.0 for Windows (Chicago, Illinois, USA).

Acknowledgments: We thank CoQuaLab (www.coqualab.it) for its technical support.

Conflict of interest: none.

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Table 1. Mean, standard deviation, median and interquartile range for age, body mass index and itraconazole plasma concentrations.

Variable	N=290			
	Mean	**Standard Deviation**	**Median**	**IQR**
Age (years)	8.56	4.36	9.00	5.00-12.00
BMI Kg/m^2	17.34	4.61	16.60	14.58-19.87
ITC C_{trough} ng/mL	579.14	713.23	306.50	91.00-781.25

List of abbreviations: N, number; IQR, interquartile range; ITC, itraconazole; C_{trough}, concentration at the end of dosing interval; BMI, body mass index.

Table 2. Number and percentage of patients for each dose regimens.

		N=290	
ITC dose	**Dose score**	**N**	**%**
1	20 t.d.	7	2.4
2	25 t.d.	4	1.4
3	30 t.d.	10	3.4
4	40 t.d.	27	9.3
5	45 t.d.	10	3.4
6	50 t.d.	40	13.8
7	60 t.d.	22	7.6
8	70 t.d.	15	5.2
9	75 t.d.	5	1.7
10	80 t.d.	20	6.9
11	100 q.d.	6	2.1
12	100+50	4	1.4
13	100 t.d.	67	23.1
14	100+150	3	1.0
15	100+200	151	5.2
16	150 t.d.	4	1.4
17	300 q.d.	5	1.7
18	200 t.d.	26	9.0

List of abbreviation: ITC, itraconazole; N, number; %, percentage; q.d., once daily; t.d., twice daily.

Table 3. Factors, in univariate and multivariate linear regression analysis, able to predict itraconazole plasma concentrations at the end of dosing interval.

	Univariate		Multivariate	
	***p* value**	**β (IC 95%)**	***p* value**	**β (IC 95%)**
Ethnicity	**0.021**	**-0.135 (-356.46; -28.99)**	**0.007**	**-0.161 (-395.04; -62.38)**
Age	0.727	-0.021 (-22.32; 15.59)		
Gender	**0.124**	**0.090 (-36.47; 299.76)**	**0.038**	**0.123 (9.60; 349.40)**
BMI	0.987	-0.001 (-18.09; 17.81)		
ITC dose	0.681	-0.024 (-21.04; 13.77)		

β: β coefficient; IC95%: interval of confidence at 95%; in bold: values with a statistically significant *p* value; ITC, itraconazole; BMI, body mass index.

Table 4. Factors, in univariate and multivariate logistic regression analyses, able to predict itraconazole prophylaxis efficacy cut-off (250 ng/mL).

	Univariate		Multivariate	
	***p* value**	**β (IC 95%)**	***p* value**	**β (IC 95%)**
Ethnicity	0.851	0.957 (0.60; 1.52)		
Age	0.378	1.024 (0.97; 1.08)		
Gender	**0.061**	**1.581 (0.98; 2.56)**	0.061	1.581 (0.98; 2.56)
BMI	0.326	1.026 (0.98; 1.08)		
ITC dose	0.257	1.029 (0.98; 1.08)		

β: β coefficient; IC95%: interval of confidence at 95%; in bold: values with a statistically significant *p* value; ITC, itraconazole; BMI, body mass index.

Table 5. Itraconazole intra- and interday accuracy and precision.

RSD: relative standard deviation.

		Precision (% RSD)	
Nominal value (µg/mL)	**Accuracy (% deviation)**	**Intraday**	**Interday**
0.10	6.23	9.12	6.34
1.50	2.30	4.30	8.70
3.00	0.14	5.72	12.01
5.00	4.60	7.86	12.64