Title

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Journal

Antimicrobial Agents and Chemotherapy, 65(4)

ISSN

0066-4804

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Publication Date

2021-03-18

DOI

10.1128/aac.02353-20

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

Variability of Hydroxy-Itraconazole in Relation to Itraconazole Bloodstream Concentrations

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ABSTRACT We analyzed the relationship between itraconazole (ITZ) and hydroxy-itraconazole (OH-ITZ) levels in 1,223 human samples. Overall, there was a statistically significant correlation between ITZ and OH-ITZ levels (Pearson's *r*, 0.7838), and OH-ITZ levels were generally higher than ITZ levels (median OH-ITZ:ITZ ratio, 1.73; range, 0.13 to 8.96). However, marked variability was observed throughout the range of ITZ concentrations. Thus, it is difficult to predict OH-ITZ concentrations based solely on ITZ levels.

KEYWORDS itraconazole, hydroxy-itraconazole, therapeutic-drug monitoring, TDM, bloodstream concentrations, bioassay, antifungal efficacy

Historically, itraconazole (ITZ) has been the preferred antifungal for treatment of most dimorphic fungal infections and some less-acute forms of aspergillosis (1–3). However, its oral bioavailability is variable and influenced by formulation, coingestion of food, and coadministration of medications that affect gastric acidity and motility (4). In addition, drug-drug interactions can affect ITZ exposure, which has been correlated with clinical outcomes (5). Consequently, therapeutic-drug monitoring (TDM) of ITZ is recommended for prophylaxis and treatment with this triazole (2, 3, 6).

Bioassays have been used for ITZ quantitation in biological fluids; however, these assays lacked sensitivity, and later studies found that correlation with drug concentrations measured by analytical assays (e.g., high-performance liquid chromatography [HPLC], liquid chromatography-mass spectrometry [LC-MS]) is variable and dependent on several factors, including the indicator organism used in bioassays (7–10). The results of bioassays are generally higher than those of HPLC or LC-MS when measured in human specimens, and levels measured by bioassay have been reported to range between 2 and 10 times higher than by the other analytical methods (7–10). This is due to the presence of hydroxy-itraconazole (OH-ITZ), an early metabolite in the metabolic pathway of ITZ, which has broad-spectrum antifungal activity and *in vitro* potency similar to those of ITZ (11). Others have suggested that the lack of correlation between ITZ levels measured by bioassay and analytical methods may be the result of the precipitation of ITZ in some bioassays due to the poor aqueous solubility of this triazole (12).

The availability of rapid, accurate, and cost-effective ITZ and OH-ITZ TDM has prompted measurement of both values in some clinical laboratories, and reporting of both levels is recommended in some treatment guidelines (1, 2, 13). The relationship of these values to one another has been examined in only a limited manner (7), and thus clinical uncertainty with interpretation remains. Prior publications have defined

Citation Wiederhold NP, Schwartz IS, Patterson TF, Thompson GR, III. 2021. Variability of hydroxy-itraconazole in relation to itraconazole bloodstream concentrations. Antimicrob Agents Chemother 65:e02353-20. https://doi.org/10.1128/AAC.02353-20.

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Received 6 November 2020
Returned for modification 15 December 2020
Accepted 9 January 2021
Accepted manuscript posted online 19 January 2021
Published 18 March 2021

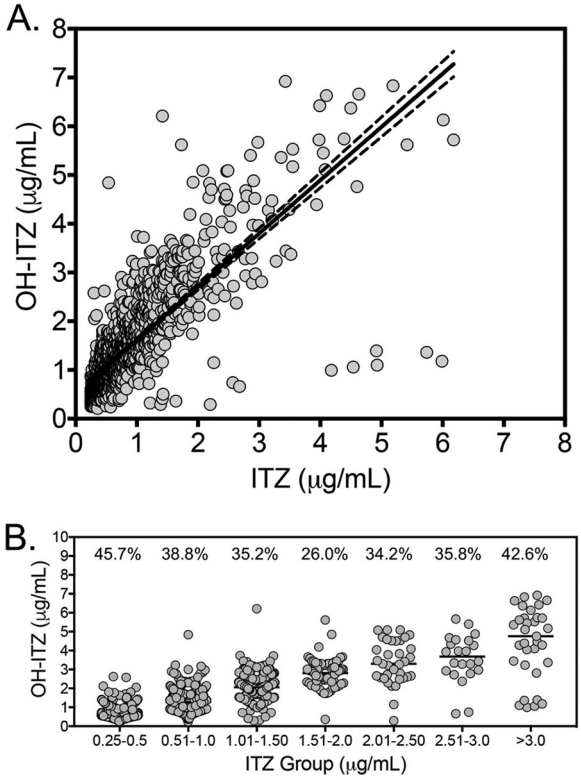

FIG 1 (A) Linear regression analysis between ITZ and OH-ITZ. ITZ and OH-ITZ were measured by validated HPLC or UPLC-MS assays, and concentrations within the analytical measurement range (0.25 to 6 μg/ml) were plotted ($n = 1,223$). (B) OH-ITZ concentrations based on different ITZ concentration levels. The percent coefficients of variation of OH-ITZ are presented above each ITZ group.

the target level of ITZ during prophylaxis as $>0.5\,\mu$g/ml, with treatment targeting a value of $>1.0\,\mu$g/ml. However, neither the target OH-ITZ level nor the target of the sum of the concentrations (ITZ plus OH-ITZ) that should be achieved to improve therapeutic outcomes has been defined. We sought to evaluate the relationship between ITZ and OH-ITZ and to summarize past studies to provide recommendations on ITZ and OH-ITZ TDM.

Paired ITZ and OH-ITZ levels from the Fungus Testing Laboratory (UT Health San Antonio, TX) were reviewed. These were measured by HPLC or ultraperformance liquid chromatography (UPLC)-MS as previously described (14, 15). Levels below or above the validated analytical measurement range for this laboratory (0.25 to 6 μg/ml) were excluded from the analysis. Linear regression analysis was used to plot ITZ versus OH-ITZ levels, and Pearson's correlation (r) was used to assess the relationship between these measurements. A P value of <0.05 was considered statistically significant. A total of 1,223 paired human measurements of ITZ and OH-ITZ were included in this analysis. Pearson's r was 0.7838 (95% confidence interval, 0.7612 to 0.8045; $P < 0.001$) (Fig. 1A), whereas the coefficient of determination was 0.6143. The median OH-ITZ:ITZ ratio was 1.73 (range, 0.13 to 8.96), the mean ratio was 1.829 ± 0.724, and 5.64% of samples had an OH-ITZ:ITZ ratio of ≤ 1. Overall, these results suggest that the values of OH-ITZ and ITZ are correlated, and OH-ITZ concentrations are usually higher than those of ITZ. These results are consistent with a previous study that included <40 samples, where the mean ratio was 1.8 ± 0.5 and the correlation coefficient was 0.96 (7). We also plotted OH-ITZ values based on ITZ concentration groups (Fig. 1B). Overall, the coefficient of variation for OH-ITZ levels varied from 26% to 45.7% and was greatest within the lowest and highest ITZ groups (45.7% for the 0.25- to 0.5-μg/ml group and 42.6% for

TABLE 1 Summary of clinical studies showing response rates in relation to ITZ and OH-ITZ levels

Study type and condition (no. of patients)	ITZ dose (mg)	Analyte and assay method	Findings (ref.)[a]
Prophylaxis			
Neutropenia (72)	100 twice daily	ITZ alone by HPLC	Fungal infections in 16/31 (52%) of patients with ITZ levels of <0.25 μg/ml vs 3/37 with levels of >0.25 μg/ml (8.1%) (23)
Neutropenia (20)	400–800 daily	ITZ alone by HPLC	Significant difference in percent ITZ concentrations of >0.5 μg/ml in patients with invasive fungal infections (median 48%) vs those without infections (median 100%); lower median ITZ levels immediately before diagnosis of infection in patients with fatal infections (0.12 μg/ml) vs those with nonfatal infections (0.69 μg/ml) (24)
Neutropenia (45)	200 twice daily	ITZ alone by HPLC	Fungal infections in 11/21 with inadequate ITZ levels (<0.25 μg/ml for 7 consecutive days) vs 4/21 with adequate levels (21)
Treatment			
Oral candidiasis (31)	200 twice daily	ITZ and OH-ITZ by HPLC	Serum ITZ levels of 1.19 vs 0.63 μg/ml in responders vs nonresponders, respectively; OH-ITZ levels of 1.38 vs 0.71 μg/ml in responders vs nonresponders, respectively (17)
Oral candidiasis (264)	200 twice daily	ITZ by HPLC	Trough ITZ of >0.5 μg/ml associated with highest rate of treatment success (20)
Aspergillosis (15)	100–400 daily	ITZ/OH-ITZ by bioassay	Mean ITZ levels of 6.1 vs 2.8 μg/ml in cures/responders vs nonresponders/failures, respectively (19)
Coccidioidomycosis (39)	200 twice daily	ITZ/OH-ITZ by bioassay	Mean C_{max} of ITZ 6.5 vs 4.0 μg/ml in responders vs nonresponders (22)

[a]C_{max}, maximum concentration of drug in serum.

the >3-μg/ml group). These results suggest that it may be difficult to accurately predict OH-ITZ levels based solely on ITZ concentrations.

Both OH-ITZ and ITZ are primarily metabolized by the cytochrome P450 3A4 isoenzyme, and the differences in the ratio of parent drug to metabolite are likely secondary to differences in CYP3A4 affinity, as has been suggested (16). Despite the correlation, we observed that there are clear outliers. These outliers may have polymorphisms within the metabolic pathway of either the parent drug or the metabolite or due to patients receiving concomitant medications that either induce or inhibit the metabolism of ITZ and/or OH-ITZ.

Past itraconazole concentration-effect studies have shown correlations between bloodstream drug concentrations and clinical efficacy (7, 17–24). These studies have focused on oral candidiasis, aspergillosis, cryptococcosis, and coccidioidomycosis (Table 1). However, not all studies have reported such a correlation (25, 26). The relationship between toxicity and ITZ concentrations is less clear. Some have suggested that concentrations of >17 μg/ml (measured by bioassay) were associated with significant toxicity, including clinical features associated with heart failure (27). The exact toxicity threshold when measured by HPLC or LC-MS is unknown but has been suggested to be $\sim$5 times lower when considering the ITZ component alone (6, 12). Current guidelines for target drug concentrations have been based on these studies, with ITZ drug levels of >0.5 μg/ml targeted during prophylaxis and ≥ 1.0 μg/ml during treatment (1, 2, 13). These recommendations have focused on ITZ concentrations and have not included OH-ITZ levels, causing uncertainty with interpretation of these values. It is unknown how many centers that measure ITZ levels also measure and report OH-ITZ levels.

In summary, although a significant correlation between ITZ and OH-ITZ bloodstream concentrations was found, variability in some samples was also observed. This suggests that OH-ITZ concentrations may not be interpolated solely based on ITZ levels. Given the sum of available data (summarized in Table 1) and an OH-ITZ:ITZ ratio of >1 in $>94\%$ of samples, it may be feasible to recommend a target ITZ concentration

of >0.5 μg/ml and combined ITZ+OH-ITZ levels of ≥1.0 μg/ml during prophylaxis and ITZ concentrations of ≥1.0 μg/ml and combined ITZ+OH-ITZ levels of ≥2.0 μg/ml during treatment. However, further studies are needed for clinical validation.

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