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CASE REPORT

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Significant elevation of free itraconazole concentration at onset of adverse effects: A case report

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Abstract

Free itraconazole and hydroxyitraconazole concentrations were markedly elevated despite almost no changes in total concentrations when itraconazole was discontinued due to adverse effects. Elevated free itraconazole concentration may have a causal relationship with the development of adverse effects.

KEYWORDS

adverse effects, free concentration, hydroxyitraconazole, Itraconazole, therapeutic drug monitoring

1 | INTRODUCTION

Only free drug contributes to efficacy and toxicity. In this case, when itraconazole was discontinued due to adverse effects, free itraconazole and hydroxyitraconazole concentrations were markedly elevated despite almost no changes in total concentrations. Elevated free itraconazole concentration may have a causal relationship with the development of adverse effects.

Itraconazole (ITCZ) is a triazole antifungal agent with broad antifungal spectrum and potent antifungal activity for various fungi such as *Candida* and *Aspergillus* spp.[1,2] This drug is metabolized to many metabolites which are then eliminated. Hydroxyitraconazole (OH-ITCZ) is a major metabolite produced by cytochrome P450 (CYP) 3A4 and has the same potent antifungal activity in vitro as the unchanged form.[3,4]

The pharmacokinetics of ITCZ and OH-ITCZ fluctuates widely due to interindividual differences such as digestibility and metabolic activity.[5,6] Therefore, therapeutic drug monitoring (TDM) is required to predict pharmacological efficacy and development of adverse events. Although the effective concentration range of ITCZ, defined as the sum of trough concentrations of ITCZ and OH-ITCZ, is recommended to be 500-2000 ng/mL or more,[7] a recent prospective study on immunocompromised patients indicated no relationship of plasma trough concentrations of ITCZ and/or OH-ITCZ with efficacy and safety.[8] The clinical significance of TDM remains controversial.

The protein binding rate of ITCZ is extremely high (99.8%), and ITCZ mainly binds albumin.[9,10] In general, only free drug unbound to plasma proteins such as albumin distributes to tissues or organs and determines efficacy or toxicity in vivo.[11,12] Monitoring free ITCZ and OH-ITCZ concentrations may be more useful to judge efficacy or toxicity than monitoring total drug concentrations.

We have recently developed and validated a sensitive and selective quantification method of total and free ITCZ and OH-ITCZ in human plasma using ultra-performance liquid chromatography coupled to tandem mass spectrometry

(UPLC-MS/MS).[13] In this report, we present a case showing marked increases in free ITCZ and OH-ITCZ concentrations despite little changes in total drug concentrations when ITCZ treatment was discontinued because of adverse effects.

2 | CASE PRESENTATION

A 41-year-old man was readmitted to Oita University Hospital for investigation of a lung abscess. The patient was diagnosed with chronic granulomatous disease, and lung and cerebral aspergillosis in his childhood. Three months before readmission, a computed tomographic (CT) scan revealed an abscess in the right thoracic wall, and intermittent percutaneous drainage was performed as an attempt to remove the abscess. However, as the abscess did not diminish in size, he was readmitted to our hospital for detailed investigations and treatment (day 1). Laboratory findings and clinical parameters on admission are shown in Table 1. His primary diseases were chronic granulomatous disease, pulmonary mycosis, and chest wall abscess.

Figure 1 shows his progress after hospitalization. From day 1, treatment with a combination of VRCZ and caspofungin (CPFG) was initiated. Despite maintaining high trough VRCZ concentration of more than 5 μg/mL while monitoring adverse effects, no sufficient clinical response was observed. Co-infection with other *Aspergillus* strains or resistance to azole antifungal drugs was suspected. Hence, samples were sent to Medical Mycology Research Center at Chiba University for identification of *Aspergillus* strains and drug susceptibility test. On day 8, VRCZ was withdrawn due to insufficient efficacy, and liposomal amphotericin B (L-AMB) was initiated instead. On day 26, *Aspergillus udagawae*, a cryptic species of *Aspergillus fumigatus*, was identified as the pathogenic fungi. Drug susceptibility test for the strain showed resistance to VRCZ; low sensitivity to L-AMB; and sensitivity to ITCZ, micafungin, and CPFG (Table 2). On day 36, the encapsulated formulation of ITCZ was added according to the result of drug sensitivity test. L-AMB was withdrawn on day 41 due to suspected adverse effects including anemia, thrombocytopenia, and hypokalemia. On day 43, the dosage form of ITCZ was switched to intravenous injection (200 mg/day).

Multiple measurements of total and free ITCZ and OH-ITCZ concentrations in plasma were started after switching to injection. The measurements were approved by the ethics committee of Oita University (approval number: 945) and started after obtaining written informed consent from the patient. These concentrations were determined using our previously developed UPLC-MS/MS method.[13] Table 3 shows the changes in trough concentrations of various forms of ITCZ and indicators of some adverse effects. The concentrations of total and free ITCZ and OH-ITCZ gradually increased over time. On day 64, total ITCZ and OH-ITCZ concentrations reached high levels of 1883.5 ng/mL and 3856.6 ng/mL, and free ITCZ and OH-ITCZ concentrations were 0.15 ng/mL and 9.43 ng/mL, respectively. On day 66, the dose of ITCZ was reduced from 200 mg/day to 150 mg/day due to mild anemia and respiratory distress. Moreover, the amount of oxygen inhaled was gradually increased to maintain SpO_2. One day later (day 67), mild elevation of hepatic function markers and aggravation of anemia were observed. Furthermore, respiratory discomfort symptoms were exacerbated, with stridor and significant decrease of SpO_2 after ITCZ injection. The above

TABLE 1 Laboratory findings and clinical parameters

Item	Value	Item	Value
Height (cm)	165.2	Blood urea nitrogen (mg/dL)	16.9
Weight (kg)	53.3	Serum creatinine (mg/dL)	0.56
Body temperature (℃)	36.8	Serum sodium (mmol/L)	136.8
Systolic blood pressure (mm Hg)	107	Serum potassium (mmol/L)	4.22
Diastolic blood pressure (mm Hg)	67	White blood cell count ($\times 10^3$/μL)	11.56
Pulse rate (bpm)	100	Red blood cell count ($\times 10^6$/μL)	3.47
SpO_2 (%)	98	Hemoglobin (g/dL)	9.9
C-reactive protein (mg/dL)	16.8	Hematocrit (%)	30.8
Serum albumin (g/dL)	2.57	PT-INR	1.12
Aspartate transaminase (U/L)	29.7	D-dimer (μg/mL)	0.74
Alanine transaminase (U/L)	8.9	pH	7.46
Alkaline phosphatase (U/L)	626	pCO_2 (mm Hg)	34.0
γ-glutamyl transpeptidase (U/L)	133.8	pO_2 (mm Hg)	87.0
Total bilirubin (mg/dL)	0.37	HCO_{3-} (mmol/L)	24.2

Abbreviations: HCO_{3-}, bicarbonate ion; pCO_2, carbon dioxide partial pressure; pO_2, oxygen partial pressure; PT-INR, international normalized ratio of prothrombin time; SpO_2, percutaneous oxygen saturation.

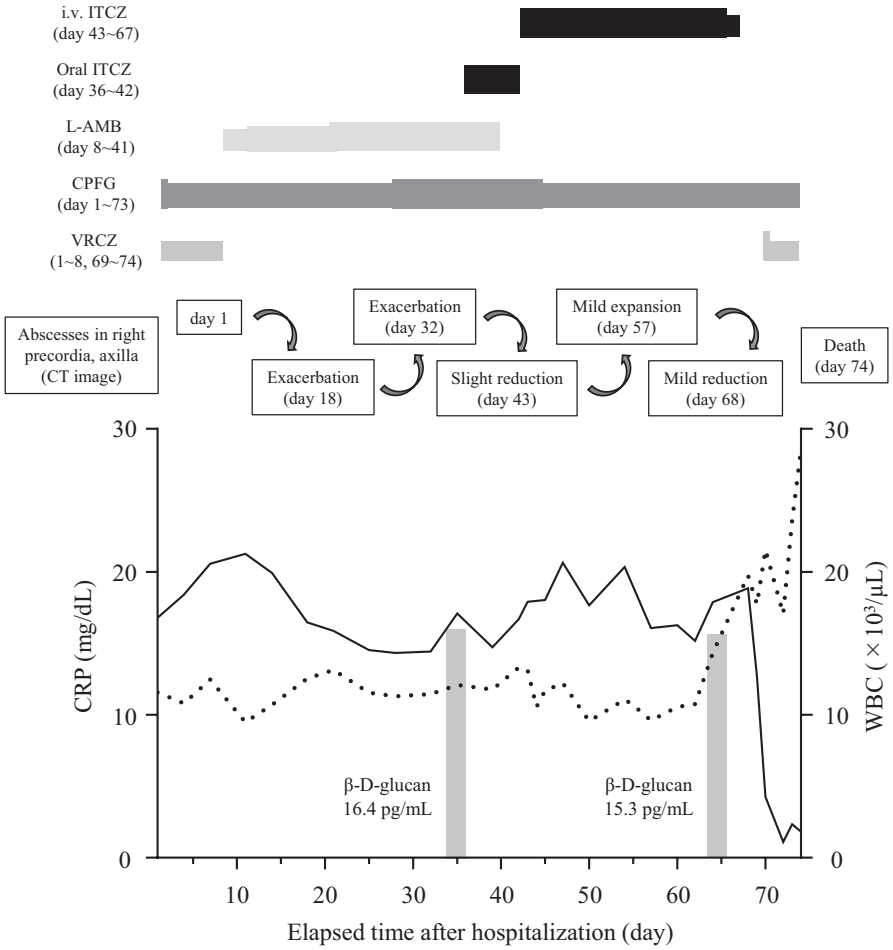

FIGURE 1 Progress after hospitalization. Solid line represents CRP levels and dotted line represents WBC counts. Iv, intravenous; ITCZ, itraconazole; L-AMB, liposomal amphotericin B; CPFG, caspofungin; VRCZ, voriconazole; CT, computed tomographic; CRP, C-reactive protein; WBC, white blood cell

TABLE 2 The result of drug sensitivity test for *Aspergillus udagawae*

Antifungal drug	MIC (μg/mL)
Micafungin	<0.015
Caspofungin	0.25
Amphotericin B	2
5-fluorocytosine	64<
Fluconazole	64<
Itraconazole	0.5
Voriconazole	4
Miconazole	2

Abbreviation: MIC, minimum inhibitory concentration.

symptoms were suspected to be caused by adverse effects of ITCZ, and the drug was discontinued on day 68. Two days after discontinuation, total ITCZ and OH-ITCZ concentrations were 1885.9 ng/mL and 3426.7 ng/mL, and free ITCZ and OH-ITCZ concentrations were 3.09 ng/mL and 19.3 ng/mL, respectively. Of note, free ITCZ and OH-ITCZ concentrations were approximately 20 and 2 times higher than those before discontinuation despite almost no changes in total ITCZ and OH-ITCZ concentrations.

3 | DISCUSSION

In a previous report, trough concentration below 500 ng/mL was associated with increased potential of breakthrough infections and significantly increased mortality in neutropenic patients receiving ITCZ for prophylaxis.[14] Additionally, ITCZ trough concentration of 1000 ng/mL or less was predictive of failed drug treatment for oropharyngeal candidiasis.[15] Although a guideline recommends concentration monitoring and careful dose adjustment when ITCZ is used for the treatment of *Aspergillus*,[16] the appropriate target trough concentration for recent *Aspergillus* strains including resistant strains is unknown.[17] In the present case, intravenous injection of ITCZ was selected to obtain higher plasma concentration, and high trough concentrations of ITCZ and OH-ITCZ were maintained. CT images on day 68 showed that the abscesses in right precordia, axilla, right lung apex,

TABLE 3 Detailed clinical parameters of the patient

Day	47[a]	54	64	66[b]	68[c]	70
Days after ITCZ initiation	5	12	22	24	26	28
ITCZ dose (mg/day)	200	200	200	150	–	–
Total ITCZ conc. (ng/mL)	–	1384.6	1883.5	–	–	1885.9
Total OH-ITCZ conc. (ng/mL)	–	2419.9	3856.6	–	–	3426.7
Free ITCZ conc. (ng/mL)	–	0.13	0.15	–	–	3.09
Free OH-ITCZ conc. (ng/mL)	–	6.23	9.43	–	–	19.3
Lowest SpO_2 per day	98	97	90	93	93	92
Highest oxygen inhalation rate (L/min)	–	–	1	2.5	15	35
Alanine transaminase (U/L)	6.5	5.1	7.7	–	12.5	19.4
Serum albumin (g/dL)	2.08	1.92	2.26	–	2.13	2.25
Total bilirubin (mg/dL)	0.67	0.94	1.02	–	1.05	0.78
Serum potassium (mmol/L)	3.11	2.78	3.44	–	3.86	4.01
Hemoglobin (g/dL)	8.9	8.6	8.2	–	6.0	8.0
Hematocrit (%)	27.8	26.1	24.9	–	18.3	24.8

Abbreviations: ITCZ, itraconazole; OH-ITCZ, hydroxyitraconazole; SpO_2, percutaneous oxygen saturation.

[a]Intravenous injection of ITCZ 200 mg/day was initiated.

[b]ITCZ dose was reduced to 150 mg/day.

[c]ITCZ was discontinued.

and mediastinum were reduced compared with day 57, confirming a certain level of response to ITCZ.

In contrast to the large number of publications on effectiveness, there is a paucity of reports indicating an association of plasma ITCZ concentration with toxicity. The safety profile of ITCZ is less favorable than fluconazole and is associated with cardiotoxicity, gastrointestinal intolerance, neuropathy, and hepatitis.[18,19] Lestner et al[20] demonstrated that plasma ITCZ concentration exceeding 17 000 ng/mL measured using a bioassay was associated with an increase in probability of drug-related toxicities such as fluid retention, gastrointestinal intolerance, and liver dysfunction. Equivalent toxic threshold for plasma concentrations measured by HPLC has not been specifically identified, but concentration measured by HPLC is known to be one-fifth of that measured by bioassay.[21] Furthermore, signs of congestive heart failure were found in 3 of 46 patients with fluid retention in the report of Lestner et al.[20] Our patient had a high serum brain natriuretic peptide level of 113.8 pg/mL on day 61, and transthoracic echocardiography found mild pericardial effusion. Hence, in this case also, congestive heart failure related to ITCZ was suspected. Additionally, when trough total ITCZ concentration increased to 1883.5 ng/mL, respiratory distress deteriorated and oxygen inhalation volume increased significantly due to reduced SpO_2 level. The patient complained of very severe respiratory distress and lassitude after injection on day 67, leading to discontinuation of ITCZ on the next day. At this time, exacerbation of cardiac dilatation, pneumonia, and progression of granulation tissue around the bronchi was observed on CT images, which would suggest a relationship of these disease states with dyspnea. However, considering the severe respiratory distress and fatigue after the ITCZ injection, these symptoms could probably be due to the adverse effects of ITCZ.

Shortly after discontinuation, the free ITCZ and OH-ITCZ concentrations were approximately 20 and 2 times, respectively, higher than those before discontinuation, despite almost no changes in total concentrations. ITCZ not only binds albumin but also alpha 1-acid glycoprotein; however, the in vitro albumin binding rate (99.8%) is higher than that of alpha 1-acid glycoprotein (80%) and plasma albumin concentration is also higher by about 50 times. Thus, albumin is speculated to play a major role in the significant increase in free ITCZ concentration despite almost no change in total concentration, probably by the following mechanisms: (a) reduced albumin level, (b) displacement, (c) post-translational modification of albumin, and (d) saturation of albumin binding ability. Each of these possibilities will be discussed. (1) ITCZ binds proteins, mainly albumin, at an extremely high rate of 99.8%.[9,10] Therefore, reduced albumin concentration could cause transient elevation of free ITCZ. However, albumin concentration did not change significantly from before to after onset of adverse effects. (2) Regarding displacement, no drug with a higher albumin binding rate than ITCZ was added. (3) Recent reports suggested that post-translational modifications of albumin such as glycation and oxidation caused elevation of free fractions of ligands.[22,23] Thus, structural alteration of albumin after

onset of adverse effects could cause elevated free ITCZ concentration. We measured the fractions of albumin isoforms by the electrospray ionization time-of-flight mass spectrometry method,[23] but found no structural alterations after onset of adverse effects (data not shown). Hence, possibilities (1) to (3) seemed to be unrelated to the increase in free ITCZ concentration. (4) Serum albumin concentration in this patient was very low (2.1 g/dL) at the time of discontinuation. Gradual elevation of total ITCZ concentration may have led to saturation of albumin binding ability. However, we were not able to verify this possibility, and this is a limitation of this case report.

Although increased free fraction of ITCZ theoretically leads to increased hepatic clearance, which would reduce plasma total ITCZ concentration, the total ITCZ concentration in our case changed slightly in spite of marked elevation of free ITCZ. We speculated that this finding was due to decreased intrinsic hepatic clearance, because hepatic clearance is determined by intrinsic hepatic clearance and free fraction of ITCZ. However, while the patient had altered systemic condition, hepatic reserve and concomitant drugs were not changed. We were not able to verify this possibility. This is another limitation of our case report. This is another limitation of our case report.

In conclusion, free ITCZ and OH-ITCZ concentrations shortly after discontinuation of ITCZ were approximately 20 and 2 times, respectively, higher than those before discontinuation, despite almost no changes in total concentrations. Thus, elevated free ITCZ concentration may have a causal relationship with the development of adverse effects. In the future, we plan to measure free ITCZ concentrations in more cases and conduct further analysis.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

RT: measured the samples, analyzed and interpreted the data, and drafted the article. YS: established the measurement method and revised the article. RT: established the measurement method and revised the article. HM, MY, KU, and KH: diagnosed the disease and treated the patients. KH, JK, and HI: provided intellectual content of critical importance and revised the article. KK: identified the strain, test drug susceptibility and revised the article. All authors approved the final version of the manuscript.

ETHICAL APPROVAL

The study was approved by the ethics committee of Oita University (approval number: 945).

PATIENT CONSENT STATEMENT

The patient provided consent for publication of this report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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