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Original Article

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ABSTRACT

Background: Itraconazole (ITCZ) is used to treat pulmonary aspergillosis, but findings regarding the range of effective plasma concentrations are often contradictory. This study attempted to determine effective plasma concentrations of ITCZ and its active metabolite hydroxyitraconazole (OH-ITCZ) by retrospectively analyzing their relationships to clinical efficacy.

Methods: The study included 34 patients with pulmonary aspergillosis treated using ITCZ (mean age, 70 years). Each patient was treated with 200 mg ITCZ once daily (mean duration of treatment: 384 days). Plasma concentrations of ITCZ and OH-ITCZ at trough levels from 7 to 889 days after the start of treatment were determined using high-performance liquid chromatography. Clinical efficacy was assessed through the improvement clinical symptoms.

Results: Fifteen patients were classified as effective group and the other 19 patients as non-effective group. Mean (\pm standard deviation) ITCZ trough plasma concentration was significantly higher in effective group (1254 ± 924 ng/mL) than in non-effective group (260 ± 296 ng/mL). Mean OH-ITCZ plasma concentration was significantly higher in effective group (1830 ± 1031 ng/mL) than in non-effective group (530 ± 592 ng/mL). Receiver operating characteristic curve analysis revealed the optimal cutoff for ITCZ trough plasma concentration was 517 ng/mL, and 86.7% of effective group showed concentrations exceeding this value. The optimal cutoff for total ITCZ + OH-ITCZ plasma concentration was 1025 ng/mL, and 93.3% of effective group showed a concentration exceeding this value.

Conclusions: Our findings indicate that effective plasma concentration ranges for the treatment of pulmonary aspergillosis begin at an ITCZ trough plasma concentration of 500 ng/mL and a total ITCZ + OH-ITCZ plasma concentration of 1000 ng/mL.

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1. Introduction

Pulmonary aspergillosis is a deep mycosis with a very high mortality rate, and has increased in prevalence in recent years [1]. Itraconazole (ITCZ) is a triazole antifungal agent with powerful anti-fungal activity against *Aspergillus* species. The absorption of orally administered ITCZ, however, varies widely between individuals. The drug is also susceptible to the effects of meals and

concomitant drugs. Accordingly, certain patients may not respond because adequate plasma concentrations are not achieved [2]. Guidelines for the management of aspergillosis from different countries [3,4] recommended administration plans based on the monitoring of ITCZ plasma concentrations, but have generally failed to specify target plasma concentrations for ITCZ. In Japan, the package insert for ITCZ recommends planning administration through monitoring of plasma concentrations when the drug is used to prevent deep mycoses in patients with a hematologic malignancy or who have received a hematopoietic stem cell transplant, both of which can involve neutropenia. However, measurement of ITCZ plasma concentrations is not covered by the national insurance scheme in Japan when the drug is used to treat pulmonary aspergillosis. In the absence of sufficient substantiating

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data, no target concentration ranges have been established for ITCZ in the treatment of pulmonary aspergillosis.

This study attempted to better determine effective plasma concentrations of ITCZ for treating pulmonary aspergillosis by retrospectively analyzing the relationships of ITCZ and hydroxyitraconazole (OH-ITCZ), an active metabolite of ITCZ (OH-ITCZ has the same antifungal activity as ITCZ [5]), plasma concentrations to clinical efficacy in patients who received ITCZ to treat pulmonary aspergillosis.

2. Patients and methods

2.1. Study patients

This study included 34 hospitalized patients or outpatients (22 men, 12 women; mean age, 70 years; range, 46–87 years) with pulmonary aspergillosis at National Hospital Organization Minami Kyoto Hospital between October 2012 and September 2016 who were treated with ITCZ and consented to participate. Each patient was treated with an oral liquid formulation, capsules, or tablets of ITCZ at a dose of 200 mg once daily. Throughout treatment, the ITCZ dose was not changed in any patient. This study was conducted with the approval (approval no. 23-5) of the ethics committee at Minami Kyoto Hospital and written informed consent to participate in this study was obtained from all participants.

2.2. Blood sampling procedures

Blood for analyzing concentrations of ITCZ and OH-ITCZ in plasma was collected just before the next dose (i.e., at trough level). After ITCZ treatment began, a sample was collected from each patient once from Days 7–889 at a time when the plasma concentration was likely in a steady state. Whole blood (3–5 mL) was collected in an ethylenediaminetetraacetic acid (EDTA)-treated blood sampling tube and promptly centrifuged for 5 min at 3000 rpm. Separated plasma was stored frozen at -80°C until just before analysis.

2.3. Plasma concentration analysis

Plasma concentrations of ITCZ and OH-ITCZ were determined using high-performance liquid chromatography (HPLC) with an internal standard (IS). Bulk ITCZ, OH-ITCZ, and IS were obtained from Janssen Pharmaceutical (Tokyo, Japan). The acetonitrile used was HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents were guaranteed reagents (Wako Pure Chemical Industries).

First, 50 μL of a methanol solution of the IS (50 w/v%) and 0.5 mL of a 1 M sodium carbonate solution were added to 0.5 mL of each plasma sample and mixed. Next, 5 mL of *t*-butyl methyl ether was added, and the resulting mixture was shaken for 10 min and centrifuged for 5 min at 3000 rpm, and the supernatant was collected. Then, 1.5 mL of a 1 M sulfuric acid solution was added to this, and the resulting mixture was again shaken for 10 min and centrifuged for 5 min at 3000 rpm. The organic layer was purged. Next, 0.6 mL of a 6 M sodium hydroxide solution was added, and the mixture was mixed. Another 4 mL of *t*-butyl methyl ether was added. The resulting mixture was shaken for 10 min and centrifuged for 5 min at 3000 rpm. The supernatant was collected and evaporated to dryness at 60°C . The residue was dissolved in 200 μL of the mobile phase described below, and 50 μL of the resulting solution was injected into the HPLC.

The HPLC system consisted of an LC-20AT pumping unit, CTO-20A column oven, and SPD-20A ultraviolet absorbance detector (Shimadzu Corporation, Kyoto, Japan). The column was an Accucore XL C18 (4.6 mm internal diameter \times 150 mm; Thermo Fisher

Scientific, Yokohama, Japan). The column was set to a temperature of 45°C . The mobile phase was a 6:4 mixture of acetonitrile and 0.02% diethylamine solution. The flow rate was 1.5 mL/min. The detection wavelength was 254 nm. The limit of detection for both ITCZ and OH-ITCZ was 1 ng/mL. Retention times were 4.0 min for ITCZ, 2.5 min for OH-ITCZ, and 5.0 min for the IS.

2.4. Evaluation of clinical efficacy

Clinical efficacy was assessed through “the guideline of anti-fungal clinical evaluation committee, Japanese Society of Chemotherapy” [6], that is, an overall evaluation of the degree of improvement in 3 parameters: chest X-ray/chest computed tomography imaging findings; clinical symptoms; and laboratory test values, including β -D glucan are assessed.

2.5. Analysis

Student's *t*-test and Welch's *t*-test were used to perform statistical analyses. The level of significance was 5%. Optimal cutoff values for determining effective plasma concentrations for pulmonary aspergillosis were calculated using receiver operating characteristic (ROC) curve analysis.

3. Results

3.1. Evaluation of patient characteristics and overall clinical efficacy

The results of an evaluation of patient characteristics and overall clinical efficacy are shown in Table 1. Overall clinical efficacy was assessed as improved in 15 patients (effective group) and as unchanged or worsened in the other 19 patients (non-effective group). An investigation of background characteristics for effective group and non-effective group revealed no significant inter-group differences in sex, mean age, mean weight, mean number of days of treatment, or mean dose per unit body weight.

3.2. Results of plasma concentration analyses

Plasma concentrations in effective group and non-effective group are shown in Fig. 1A and 1B. ITCZ and OH-ITCZ plasma concentrations were widely distributed, ranging from 17 to 3547 ng/mL in effective group and 30–4203 ng/mL in non-effective group. Mean (\pm standard deviation) trough ITCZ plasma concentration in effective group was 1254 ± 924 ng/mL, 4.8-fold higher than the mean concentration of 260 ± 296 ng/mL in non-effective group. This inter-group difference was significant. Mean OH-ITCZ plasma concentration determined concurrently was 1830 ± 1031 ng/mL in effective group, 3.5-fold higher than the concentration of 530 ± 592 ng/mL in non-effective group. This inter-group difference was again significant.

Total ITCZ + OH-ITCZ plasma concentrations are shown in Fig. 1C. Total ITCZ + OH-ITCZ plasma concentrations were widely distributed in effective group and non-effective group, ranging from 47 to 7750 ng/mL. Mean total ITCZ + OH-ITCZ plasma concentration was 3025 ± 1936 ng/mL in effective group, 3.8-fold higher than the concentration of 791 ± 884 ng/mL in non-effective group. This inter-group difference was significant.

3.3. ROC curve analysis of ITCZ trough plasma concentrations and total ITCZ + OH-ITCZ plasma concentrations

ROC curves of ITCZ trough plasma concentrations and sum ITCZ and OH-ITCZ plasma concentrations are shown in Fig. 2. The optimal cutoff ITCZ trough plasma concentration was 517 ng/mL,

Table 1

Clinical efficacy and backgrounds in patients who received ITCZ treatment.

| | Effective group (n = 15) | Non-effective group (n = 19) | P-value |
|---------------------------------|-----------------------------|---------------------------------|---------|
| Male/Female | 7/8 | 14/5 | 0.16 |
| Mean age (years) | 70 ± 10 | 70 ± 8 | 0.89 |
| Mean weight (kg) | 50.1 ± 12.2 | 54.6 ± 11.0 | 0.17 |
| Mean administration days (days) | 65.3 ± 94.6 | 178.9 ± 250.4 | 0.11 |
| Mean dosage per weight (mg/kg) | 4.3 ± 1.0 | 3.8 ± 0.9 | 0.14 |

The values are mean ± standard deviation.

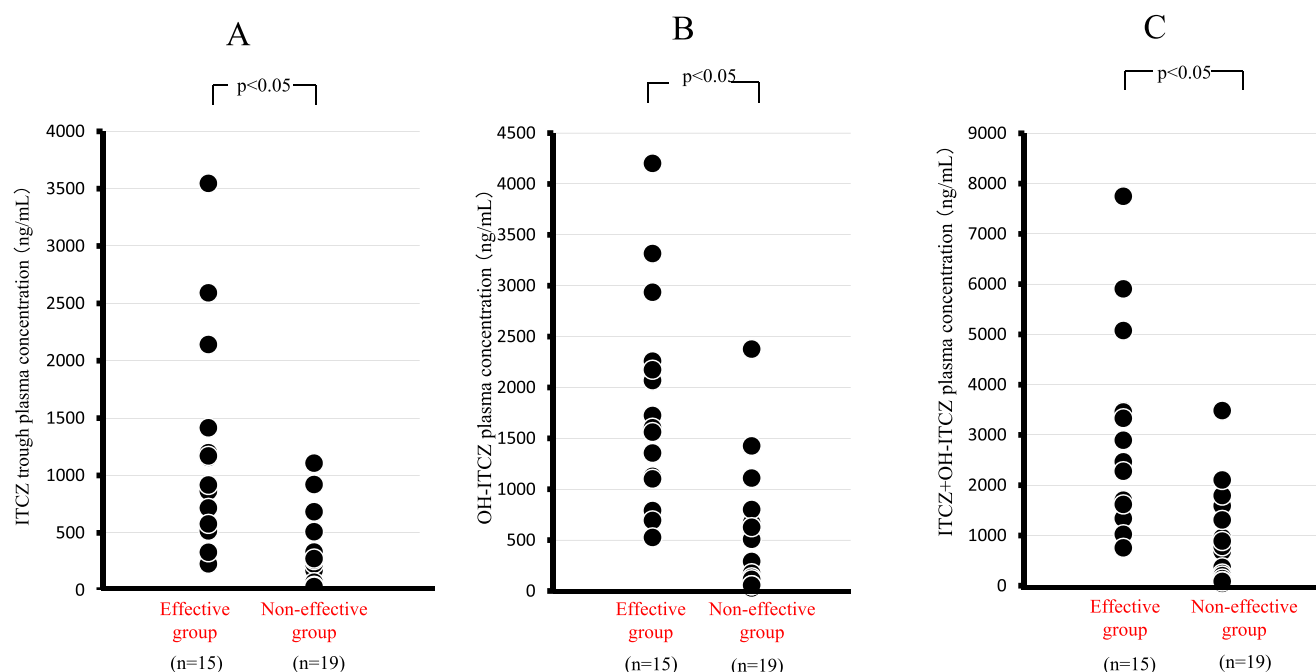


Fig. 1. Plasma concentrations of ITCZ and OH-ITCZ, plasma concentrations of ITCZ and OH-ITCZ, and total plasma concentration of ITCZ + OH-ITCZ in effective group and non-effective group. A) Mean (±standard deviation) ITCZ trough plasma concentration was 1254 ± 924 ng/mL in effective group and 260 ± 296 ng/mL in non-effective group. B) Mean OH-ITCZ plasma concentration was 1830 ± 1031 ng/mL in effective group and 530 ± 592 ng/mL in non-effective group. C) Mean total ITCZ + OH-ITCZ plasma concentration was 3025 ± 1936 ng/mL in effective group and 791 ± 884 ng/mL in non-effective group.

and the optimal cutoff total ITCZ + OH-ITCZ plasma concentration was 1025 ng/mL.

Areas under the ROC curves were 0.91 for ITCZ trough plasma concentrations and 0.90 for the sum of ITCZ and OH-ITCZ plasma concentrations.

3.4. Comparison of effective group and non-effective group for cutoff levels set at an ITCZ trough plasma concentration of 500 ng/mL and a total ITCZ + OH-ITCZ plasma concentration of 1000 ng/mL

Results from ROC curve analysis were used for a further analysis in which ITCZ trough plasma concentrations ≥500 ng/mL and total ITCZ + OH-ITCZ plasma concentrations ≥1000 ng/mL were considered as effective plasma concentrations (Table 2). Patients with ITCZ trough plasma concentrations ≥500 ng/mL accounted for 86.7% of effective group, compared with only 21.1% of non-effective group.

Patients with total ITCZ + OH-ITCZ plasma concentrations ≥1000 ng/mL accounted for 93.3% of effective group, compared with only 21.1% of non-effective group.

4. Discussion

Effective trough plasma concentrations of ITCZ suggested in the literature include ≥350 ng/mL for prophylaxis against fungal

infections [7], ≥500 ng/mL for prophylaxis and 1000–2000 ng/mL for treatment [8], and ≥1000 ng/mL for total ITCZ + OH-ITCZ plasma concentration in prophylaxis against pulmonary aspergillosis for high-risk neutropenic patients [9].

Most relevant papers have proposed concentration standards for prophylaxis, and the findings of our ROC curve analysis in Japanese patients with pulmonary aspergillosis suggest that comparable concentration standards apply for therapeutic use. The ITCZ concentration ≥500 ng/mL and total ITCZ + OH-ITCZ concentration ≥1000 ng/mL that we determined based on an area under the ROC curve ≥0.7 represent target trough concentration ranges for therapeutic purposes.

While duration of antifungal treatment for pulmonary aspergillosis should be set to at least 2 weeks, the condition of the patient should be checked every 1–3 months, with treatment discontinuation considered if test findings and clinical symptoms improve [3]. In situations requiring long-term use of an antifungal agent, intravenous administration is expensive and burdensome on the patient, so oral antifungal agents are often selected for long-term treatment [10]. ITCZ and total ITCZ + OH-ITCZ plasma concentrations as determined in this study were distributed over a wide range, despite the strict uniform dose of 200 mg/day for oral ITCZ, because liquid formulation, capsules, or tablets were administered to patients with pulmonary aspergillosis. Since ITCZ, as a weak

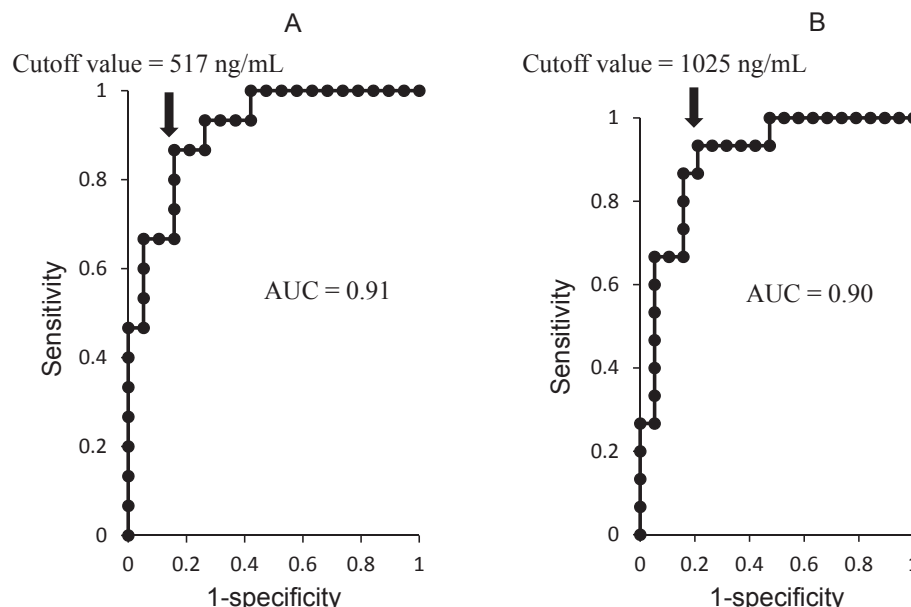


Fig. 2. ROC curves for plasma concentrations of ITCZ and of ITCZ + OH-ITCZ. A) ROC curves for trough plasma concentration of ITCZ. The optimal cutoff value is calculated as 517 ng/mL. Sensitivity and specificity at a cutoff value of 517 ng/mL are 86.7% and 84.2%, respectively. B) ROC curves for total plasma concentrations of ITCZ + OH-ITCZ. The optimal cutoff value is calculated as 1025 ng/mL. Sensitivity and specificity at a cutoff value of 1025 ng/mL are 93.3% and 78.9%, respectively.

Table 2
The relations of plasma concentrations and the clinical efficacy.

| | Effective group (n = 15) | Non-effective group (n = 19) |
|-------------------------------------|-----------------------------|---------------------------------|
| ITCZ trough plasma concentration | | |
| ≥500 ng/mL | 13 (86.7%) | 4 (21.1%) |
| <500 ng/mL | 2 (13.3%) | 15 (78.9%) |
| ITCZ + OH-ITCZ plasma concentration | | |
| ≥1000 ng/mL | 14 (93.3%) | 4 (21.1%) |
| <1000 ng/mL | 1 (6.7%) | 15 (78.9%) |

base, shows differences in dissolution at different gastric pH levels, these wide distributions may have been due to the concomitant use of gastric secretion inhibitors [11,12], reduced gastric acid secretions due to malnutrition or advanced age [13], and other factors that varied widely among patients to reduce absorption via the gastrointestinal tract. Given that the mean age of the study population was high, at 70 years, age-related reduced gastric acid secretions and associated decreases in gastrointestinal absorption may indeed have contributed to the wide distribution of plasma concentrations. The reason why the blood concentration of the effective group and the non-effective group differed even though there was no difference in the patient background was considered to be the result of the combined involvement of the above.

The national insurance scheme in Japan does not cover monitoring of ITCZ plasma concentrations for patients on ITCZ for the treatment of pulmonary aspergillosis. Our finding, however, that some patients failed to achieve sufficiently high ITCZ and total ITCZ + OH-ITCZ plasma concentrations despite receiving ITCZ at 200 mg once daily as recommended by the package insert stresses the importance of an administration plan based on monitoring of plasma concentrations when ITCZ is used for therapeutic purposes, as for prophylaxis against deep mycoses in patients with hematologic malignancy or who have received hematopoietic stem cell transplant, both of which can involve neutropenia.

In this study, there was no difference in the area under the ROC curve between the case where ITCZ plasma concentration alone

was used as the index and the case where total ITCZ + OH-ITCZ plasma concentration was used as index. Therefore, it was considered that monitoring the concentration of ITCZ alone could sufficiently reflect the effectiveness.

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Conflicts of interest

None.

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