# Population pharmacokinetics of itraconazole in Japanese patients with invasive fungal peritonitis

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Severely ill patients are frequently at risk of developing fungal infection. Itraconazole (ITCZ), a triazole antifungal agent, is used for the treatment of candidiasis, aspergillosis, cryptococcosis. Correlation of pharmacokinetic and pharmacodynamic (PK-PD) parameters with the *in vivo* bactericidal action of antimicrobial agents has progressed markedly in recent years. However, the optimal dosage of antifungal agents based on PK-PD properties has not been clearly established. In this study, we performed a population pharmacokinetic analysis of ITCZ after infusion or oral administration of ITCZ in Japanese 51 patients with fungal infections. The population pharmacokinetic analysis was performed using NONMEM software. The population mean clearance (CL; liter/h) was estimated as 5.15－0.0673×(Age－62) L/h, the population mean volume of distribution (V; liter) was determined as 878L and the bioavailability (F) was determined as 0.665.

## Introduction

The clinical efficacy and safety of intravenous itraconazole (ITCZ) were suggested in the management of proven and presumed candidiasis including *Candida glabrata* in non-neutropenic patients[1]. Since the 1990's, in the area of antimicrobial research, rapid advances have been made in both pharmacokinetic (PK) studies, including maximum blood concentration ($C_{max}$), area under the plasma concentration-time curve (AUC), and elimination half-life ($t_{1/2}$), and pharmacodynamic (PD) studies, including minimum inhibitory concentration (MIC) and time-killing curve. Such advances have greatly contributed to the development of proper therapies for infections and the development and marketing of novel antimicrobial agents. One notable achievement in this area was the identification of PK-PD parameters correlated with *in vivo* bactericidal actions of an-

timicrobial agents, such as $C_{max}$/MIC or $AUC_{0\sim24h}$/MIC for aminoglycosides and fluoroquinolones, and the percentage ratio of time above MIC to dosing interval (%t＞MIC) for $\beta$-lactams [2].

These parameters have enabled prediction of the therapeutic effects of certain antimicrobial agent to some degree, based on pharmacokinetic characteristics as represented by parameters such as $C_{max}$, AUC, and $t_{1/2}$, which are calculated from mean blood concentrations, and pharmacodynamic characteristics represented by parameters such as breakpoint value, $MIC_{50}$, and $MIC_{90}$.

On the other hand, in the field of antifungal research, evaluation of the optimal dosage based on PK-PD has yet to be sufficiently established. Louie *et al.*[3] first examined the impact of fluconazole dose fractionation and demonstrated that similar outcomes were observed whether the dose was administered as a single bolus or as two or three smaller doses. These observations suggest that the treatment outcome is affected by not the dosing interval, but by AUC. After this confirmation, Andes *et al.* investigated the relationship between efficacy and PK-PD parameter of antifungal drugs by dose fractionation studies[4~9]. These studies considered the importance of the AUC/MIC parameter for triazoles (fluconazole and ravuconazole)[4,8]. Conversely, the importance of the peak/MIC parameter has been confirmed for polyene and echinocandin[6,7]. In the investigations with flucytosine, %t＞MIC was the parameter most closely associated with efficacy[5]. Data from these *in vivo* studies can be used to examine relationships between outcome and parameter by expressing each dosing regimen as a pharmacodynamic parameter. However, compared to antibiotics, optimal dosage based on PK-PD of antifungal agents has not been applied to clinical practice and has yet to be sufficiently reported. ITCZ covers a broad antifungal spectrum, including non-*albicans Candida* and *Aspergillus* spp., and is effective for tissue transition as well as being affordable[9~13]. This agent is well tolerated and highly efficacious, as the main metabolite, hydroxy-itraconazole, also displays considerable antifungal activity. In particular, intravenous formulation of ITCZ could be used in a wide range of patient populations, including those with severe infectious complications or requiring intensive care, thanks to the improved bioavailability.

ITCZ have three type formula, capsule, solution, and injection. However, little information is available about the population pharmacokinetic (PPK) of ITCZ. In an effort to meet this need, we performed the clinical study reported here to obtain the PK parameters for ITCZ.

## I. Materials and Methods

### Subjects

All of the patients admitted to Aichi Medical University Hospital who were treated with ITCZ 51 injections changed to 36 oral for fungal infections and had blood samples taken to monitor ITCZ concentrations were included in the study. ITCZ was administered by infusion as a 40%

hydroxypropyl-$\beta$-cyclodextrin solution in water for injection. After an initial loading period lasting 2 days (200 mg every 12 h×4 times), 200mg ITCZ was infused as a maintenance dose for 60 min every 24 h in all patients. We are analyzed PPK of 236 plasmas ITCZ in 51 patients changed to 36 oral patients. Relevant clinical background and laboratory data were collected at appropriate intervals during the ITCZ treatment. We also excluded patients for whom the laboratory data needed for the present study was lacking.

**Population pharmacokinetic modeling**

The PPK analysis of ITCZ was performed using the non-linear mixed effect model (NONMEM®) software package, version 7.2.0[14]. The first order conditional estimation with interaction (FOCE-I) algorithm was used for parameter estimation. Conditional weighted residual (CWRES) was used Bootstrap resampling[15] was performed using the MULTTEST procedure of SAS software (SAS Institute Inc.). The concentration-time courses were described by use of a one-compartment model (in accordance with the available post-distribution phase data) with infusion or oral administration. Fixed- and random-effect parameters were estimated by use of the NONMEM program. The basic pharmacokinetic parameters of clearance (CL), the volume of distribution (V) and the bioavailability rate constant (F) corresponding to the proposed model were determined for each patient.

In the first phase of the analysis, a basic model with no covariates on CL, V or F was used. Exponential inter-individual variability models were invoked for each CL and V. The residual variability was determined by using an exponential error model or combined exponential and additive error model.

In the second phase of the analysis, several covariates were considered to improve the model significantly if the decrease in the objective function value (OFV) was $>3.84$ ($p<0.05$). The covariate with the greatest decrease in the OFV was added to the base model and the entire procedure was repeated until no further improvement could be obtained. All covariates (sex, age, body weight, height) that were included or selected were considered in view of their biological plausibility. Basic goodness-of-fit plots including population prediction (PRED) and individual predictions (IPRED) vs. observed concentrations (DV), as well as, conditional weighted residual (CWRES) vs. PRED and time after first dose were used for modeling diagnostic purposes. The stability and performance of the final population pharmacokinetic model were assessed using an internal validation method which involved non-parametric bootstrapping with re-sampling ($n=1000$). The model for which the probability of a successful bootstrap run was more than 90% and the parameter estimates were comparable was defined as the robust model. The model was judged on the basis of the goodness-of-fit plots, 95% confidence interval of the parameter estimate, and the likelihood ratio test.

**Evaluation of clinical response**

The subject were patients of critical care department, 18 years old or older, who received a diagnosis as invasive fungal peritonitis according to the "Guidelines for the Diagnosis/Treatment of Deep Mycosis in 2007"[16]. Clinical response was determined at the end of therapy by the physicians in charge and then confirmed by the experts. Patient response to therapy was classified as follows. Clinical outcomes were classified as either cure (eradication during treatment of the organism from specimens and/or decreased $\beta$-(1,3)-D-glucan value and improvement in clinical symptoms) or failure (no change, an increase in the amount of organism in specimens, increased $\beta$-(1,3)-D-glucan value, and a lack of improvement in clinical symptoms).

**Susceptibility testing**

Antifungal susceptibility testing of isolates of *Candida* species was performed in exact accordance with the reference broth microdilution method described by the CLSI (Clinical and Laboratory Standards Institute), document M27-A3[17]. Standard powders of ITCZ (Janssen Research Foundation, Beerse, Belgium) were obtained from the manufacturer and diluted in distilled water or dimethyl sulfoxide. Frozen microdilution panels, containing serial two-fold dilutions made in RPMI 1640 medium (Invitrogen, Tokyo, Japan) buffered to pH 7.0 with 0.165 M MOPS buffer (Wako Pure Chemical Industries, Osaka, Japan) for ITCZ were prepared in a single lot at the laboratory of BML Incorporation (Tokyo, Japan), and stored at −80°C until use. Final concentrations of ITCZ were 0.016–16 $\mu$g/mL. The final concentration of the solvent did not exceed 1% in any of the wells. Drug-free growth control wells and drug- and yeast-free sterility check wells were included. The MIC for ITCZ was defined as the lowest concentration showing 100% inhibition of growth compared with the drug-free control. Approval for this trial was received from the Clinical Research and Ethics Committee of the SRNJ. Number of the SRNJ is 0001.

## II. Results

The PPK analysis was performed using data 236 plasma ITCZ concentrations collected from 51 adult patients. The plot of time versus total plasma ITCZ concentration is shown in Fig. 1. Descriptive statistics are presented in Table 1. The pharmacokinetics of ITCZ was well-described using the one-compartment model, which included the inter-individual variability of CL and V. Residual variability was modeled using exponential error. In the covariates selection, CL was influenced by age, and V had no statistically significant covariate. The final model and its parameter estimates are shown in Table 2 and Eq. 1.

**Fig. 1. Plot of total plasma concentrations of itraconazole after first dose in patients.**

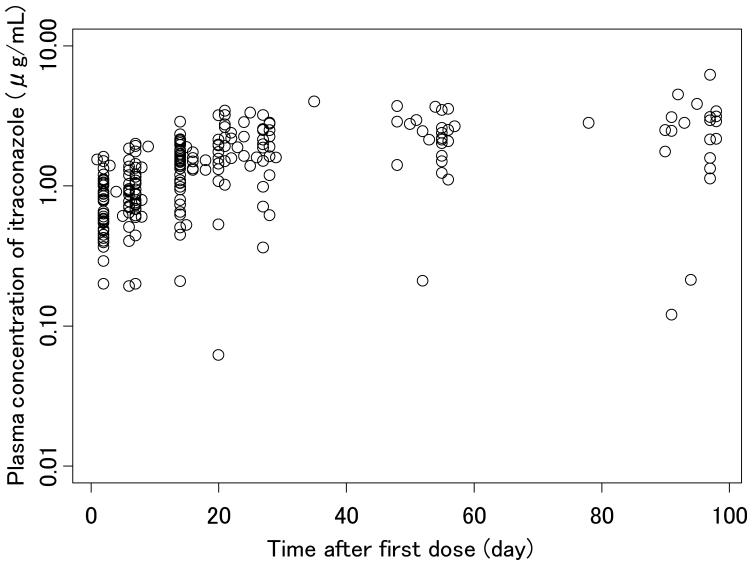



**Table 1. Demographic of population pharmacokinetics in patients.**

| | | | | |
|---|---|---|---|---|
| Sex(man/woman) | 51 | | (33/18) | |
| Age(years) | 58.14 | ± | 15.67 | (19-79) |
| Body weight(kg) | 50.91 | ± | 10.55 | (31.8-74.5) |
| Height(cm) | 158.43 | ± | 10.61 | (135-176.6) |
| Dosage | 186.36 | ± | 34.02 | (100-200) |

Arithmetic mean ± standard deviation (minimum-maximum)

$$CL=[\theta CL+(Age-62)\times\theta\ age_{(CL)}]*e^{\eta CL}$$

$$V2=\theta V2*e^{\eta V2} \quad (1)$$

$$F=\theta F$$

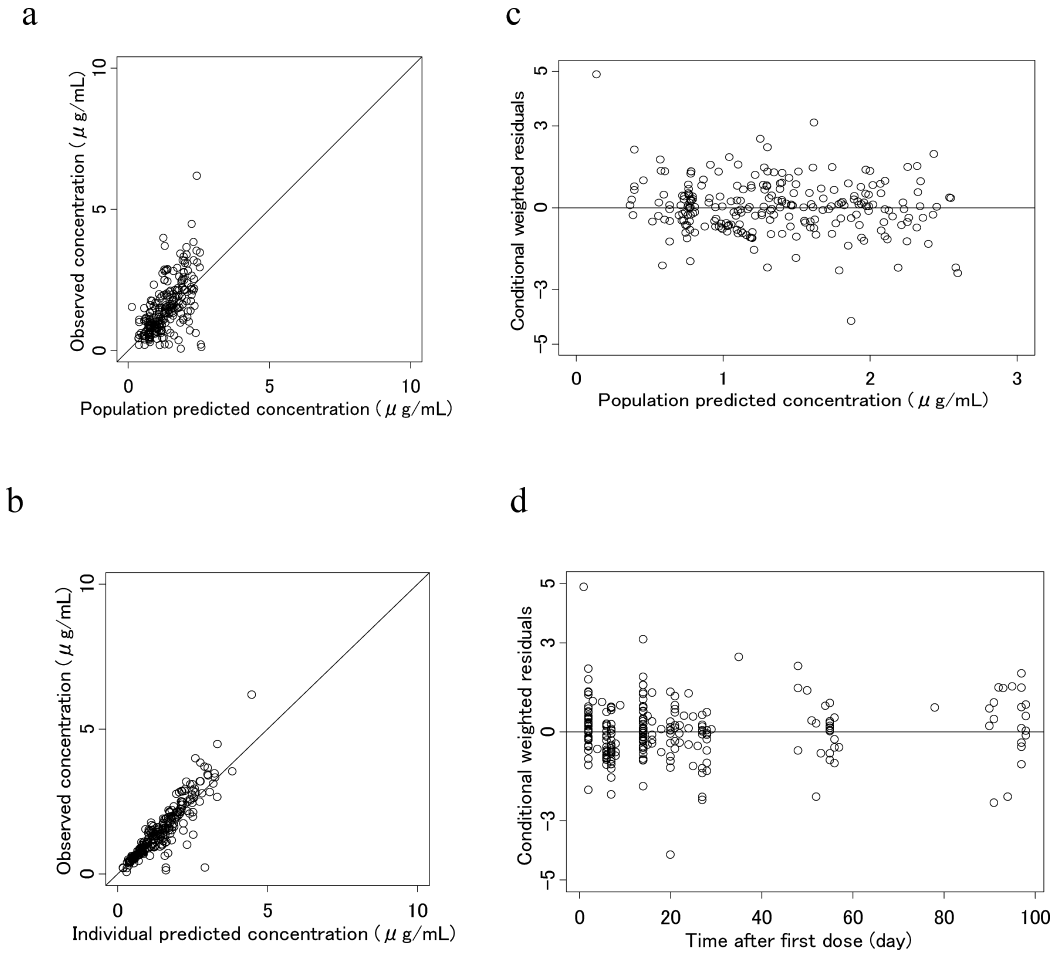
The goodness-of-fit plots of the final model are shown in Fig. 2. The concentrations predicted using the model and the individual predicted concentrations were consistent with the observed concentrations. The plots for CWRES vs. PRED and time after first dose presented good distribution around 0. The results of the bootstrap validation are shown in Table 2. The success rate was 92.7%, and the estimated population values and the average of the bootstrap results were fairly consistent, which suggested good stability in parameter estimates.

**Susceptibility to ITCZ**

All 16 in 51 patients available isolates were tested for ITCZ susceptibility in this study, and

**Fig. 2. Goodness-of-fit plots of the final population pharmacokinetic model for itraconazole.**

a. The population predicted concentrations versus the observed concentrations.
b. The individual predicted concentrations versus the observed concentrations.
c. The population predicted concentrations versus the conditional weighted residuals.
d. Time after first dose versus the conditional weighted residuals.




the results are shown in Table 3. Data are reported as the MIC range in 4 species of *Candida*. Clinical cure rate was 81.25% and the population comprised 13 responders and 3 non-responders. Non-responders showed higher MICs.

## III. Discussion

This analysis has presented a PPK model that adequately describes the pharmacokinetics of ITCZ in candidiasis. Although two-compartment models are used for many antibiotics with short infusions, a one-compartment model analysis was adopted in this study. The two-compartment model is not important for clinical dosage regimens, as shown by the method of Sawchuk-

**Table 2. Parameter estimates from the final PPK model and results of the bootstrap analysis.**

| Parameter | | Estimate | Standard error | 95%CI | Bootstrap estimate | Bootstrap standard error | Bootstrap 95% CI |
|---|---|---|---|---|---|---|---|
| **Population mean** | | | | | | | |
| CL ( L/h ) | $\theta_1$ | 5.15 | 0.421 | 4.33 - 5.98 | 5.14 | 0.446 | 4.42 - 6.12 |
| θ age (CL) | $\theta_4$ | -0.0673 | 0.0347 | -0.135 - 0.000797 | -0.0672 | 0.0350 | -0.137 - 0.00212 |
| V (L) | $\theta_2$ | 878 | 85.2 | 711 - 1040 | 889 | 81.2 | 733 - 1050 |
| F | $\theta_3$ | 0.665 | 0.0692 | 0.529 - 0.800 | 0.662 | 0.0730 | 0.522 - 0.804 |
| **Inter-subject variability** | | | | | | | |
| $\omega^2_{CL,CL}$ | | 0.254 | 0.112 | 0.0335 - 0.474 | 0.471 | 0.127 | 0.244 - 0.707 |
| $\omega^2_{V,V}$ | | 0.315 | 0.126 | 0.0687 - 0.562 | 0.541 | 0.121 | 0.312 - 0.749 |
| **Intra-individual variability** | | | | | | | |
| $\sigma^2$ (exponential error) | | 0.0728 | 0.0174 | 0.0387 - 0.107 | 0.269 | 0.0322 | 0.211 - 0.336 |

**Table 3. The relationship between MIC of ITCZ against clinical isolates and clinical effect.**

| Clinical Efficacy | Causative organism | MIC of ITCZ (μg/mL) |
|---|---|---|
| Effective | *Candida parapsilosis* | 0.125 |
| | *Candida parapsilosis* | |
| | *Candida albicans* | 0.25 |
| | *Candida albicans* | |
| | *Candida albicans* | 0.5 |
| | *Candida albicans* | |
| | *Candida parapsilosis* | |
| | *Candida tropicalis* | |
| | *Candida albicans* | 1 |
| | *Candida albicans* | |
| | *Candida albicans* | |
| | *Candida glabrata* | 2 |
| | *Candida albicans* | |
| Non-effective | *Candida tropicalis* | 2 |
| | *Candida albicans* | |
| | *Candida glabrata* | 4 |

ITCZ: itraconazole

Zaske[18] for minimization of blood loss is critical. In this study, CL of ITCZ was adjusted for age. CL is the most important factor in determining the daily dose of anti-fungal agents. The results of the bootstrap analysis confirmed the robustness of the final parameter estimates and the standard errors from the covariance step. The population parameter estimates obtained from the final model and mean of the bootstrap replicates are very similar, and the standard errors were also comparable. Therefore, a linear one-compartment model was population pharmacokinetic analysis of ITCZ because no bias was observed in the goodness-of-fit plots obtained using final model.

The importance of the AUC/MIC parameter has been confirmed on both fluconazole and a new triazole, ravuconazole[4,8]. These observations suggest that pharmacodynamic parameters associated with efficacy are similar within the azole class antifungal agents, as has been described for various antibacterials. Thus, the rate and extent of fungal killing depends upon drug trough concentration with these agents. This is very important and useful for dosing strategy to achieve the highest possible dose without causing toxicity. However, the time-course of blood concentration and MIC of an antifungal agent against the infecting fungus actually exhibit different distri-

butions depending on the individual case.

We calculated by PPK of AUC/MIC and $C_{max}$/MIC, %t＞MIC, trough/MIC, PK-PD theory, but we could not get a cut off value. In the future, there is a pressing need for antifungal therapies to consider factors such as the risk of breakthrough fungal infections and breakpoints for antifungal agents based on PK-PD analyses.

According to document M27-S3 from the Clinical and Laboratory Standards Institute, a guideline widely used for MIC breakpoints of antifungal agents, the breakpoints for ITCZ are: ≤0.125 μg/mL, susceptibility (S); 0.25–0.5 μg/mL, susceptibility-dose/delivery dependent (S-DD); and ≥1 μg/mL, resistance (R). In our study, however, an MIC of 4 μg/mL was observed in non-responders, whereas an MIC of 2 μg/mL was observed in both responders and non-responders (Table 3). We also retrospectively reported the association of both antifungal susceptibility judged by CLSI breakpoints and clinical efficacy in 16 patients with invasive fungal peritonitis treated by injectable ITCZ. Clinical success and failure were obtained in cases of ITCZ MIC ≦1 μg/mL and ≧4 μg/mL, respectively. We conclude we should re-consider CLSI breakpoints on ITCZ[9,19]. In this results, we supported our study. Since the antifungal effects of azole antifungal agents are dependent on MIC, the relationship between MIC and clinical effect was also investigated. MIC values were 0.125–2 for responders (n＝13), compared with 2–4 for non-responders (n＝3) (Table 3). Analysis of the relationship between MIC and clinical effect shows that, under the MIC of 2, efficacy rate was approximately 87%. Four patients showed MICs of 2 μg/mL. This result is comparable with the clinical efficacy of 81.25% (13 of 16 patients) obtained against *Candida* spp. isolated from peritoneal fluid samples of mycotic peritonitis patients receiving ITCZ at 200 mg/day.

Based on our results, breakpoints for ITCZ should be considerded to: ≤1 μg/mL, S; 2 μg/mL, S-DD; and ≥4 μg/mL, R. We will re-analyze PK-PD by this PPK for getting an appropriate breakpoints.

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**Transparency declarations**

The authors state that they have no conflicts of interest with the subject matter discussed in this article.

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