

# Effect of itraconazole on the concentrations of tacrolimus and cyclosporine in the blood of patients receiving allogeneic hematopoietic stem cell transplants

Miho Nara · Naoto Takahashi · Masatomo Miura ·  
Takenori Niioka · Hideaki Kagaya · Naohito Fujishima ·  
Hirobumi Saitoh · Yoshihiro Kameoka ·  
Hiroyuki Tagawa · Makoto Hirokawa · Kenichi Sawada

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## Abstract

**Purpose** The purpose of this study was to investigate the interactions of itraconazole (ITCZ) with orally administered calcineurin inhibitors (CNIs) in Japanese allogeneic hematopoietic stem cell transplant (HSCT) recipients.

**Methods** Sixteen HSCT patients (8 patients each receiving tacrolimus or cyclosporine) were enrolled. An ITCZ oral solution was administered from day 30 after the initiation of ITCZ administration as a loading dose. Before the co-administration of ITCZ and CNI and 1 week daily thereafter, whole blood ITCZ and CNI (tacrolimus or cyclosporine) concentrations were measured in samples taken just before ( $C_{0h}$ ) and 2 h ( $C_{2h}$ ) after CNI administration.

**Results** The median dose-adjusted  $C_{0h}$  values of tacrolimus and cyclosporine on day 7 after the start of ITCZ co-administration were 5.6- and 2.7-fold higher, respectively, than the corresponding values obtained before the initiation of ITCZ treatment. On day 7 after ITCZ treatment, the mean single dosages of tacrolimus and cyclosporine were reduced to 33.7 and 66.5 % of the dosages before ITCZ co-administration, respectively, to adjust the CNI target

concentration. Although ITCZ co-administration did not alter the dose-adjusted  $C_{0h}$  values of tacrolimus in a patient with a *CYP3A5\*1/\*1* allele, it did change this value of tacrolimus in patients with *CYP3A5\*3* alleles. However, in patients receiving cyclosporine, no such tendency was observed.

**Conclusion** The magnitude of the interaction between orally administered tacrolimus and ITCZ was significantly greater than that between cyclosporine and ITCZ. Prospective analysis of the *CYP3A5* polymorphism may be important to ensure safe and reliable immunosuppressive therapy with tacrolimus in patients treated with ITCZ.

**Keywords** Itraconazole · Tacrolimus · Cyclosporine · Blood concentration · Allogeneic hematopoietic stem cell transplantation

## Introduction

The clinical management of orally or intravenously administered calcineurin inhibitors (CNIs), such as tacrolimus or cyclosporine, which are widely used immunosuppressive agents in hematopoietic stem cell transplantation (HSCT) recipients, is complicated by their narrow therapeutic range and their inter- and intra-individual variable pharmacokinetics [1]. Given that CNIs are substrates of cytochrome P450 (CYP) 3A4/5, which is expressed in the small intestine and hepatocytes, the variability in their pharmacokinetics, in particular, their bioavailability, has been attributed to individual differences in CYP3A expression [2–4].

The antifungal agent itraconazole (ITCZ) is routinely used as prophylaxis against *Aspergillus* infection and often administered to HSCT recipients receiving CNIs. In several multicenter, prospective clinical trials, the elimination of

M. Nara · N. Takahashi (✉) · H. Saitoh · Y. Kameoka ·  
H. Tagawa · K. Sawada  
Department of Hematology, Nephrology, and Rheumatology,  
Akita University Graduate School of Medicine, 1-1-1 Hondo,  
Akita City, Akita 010-8543, Japan  
e-mail: naotot@doc.med.akita-u.ac.jp

M. Miura · T. Niioka · H. Kagaya  
Department of Pharmacy, Akita University Hospital, Akita, Japan

N. Fujishima  
Division of Blood Transfusion, Akita University Hospital, Akita,  
Japan

M. Hirokawa  
Clinical Oncology Center, Akita University Hospital, Akita, Japan

*Aspergillus* infection in allogeneic stem cell transplant recipients was achieved using ITCZ prophylaxis. A significant difference was observed in the incidence of *Aspergillus* infection between prophylaxis with ITCZ and that with fluconazole, particularly from day 30 after HSCT [5, 6]. However, in both in vitro and in vivo studies, ITCZ potently inhibited the activity of CYP3A4 [7–13]. Therefore, the blood concentration of CNIs may increase on their co-administration with ITCZ. In contrast, ITCZ is not a substrate of CYP3A5 [10, 14]. Although ITCZ may inhibit CYP3A5 in vitro [15], whether it inhibits CYP3A5 in vivo is unknown.

Polymorphic expression in the liver and small intestine of polymorphic forms of CYP3A5 is strongly correlated with the 6986A > G single nucleotide polymorphism (SNP) within intron 3 of CYP3A5, which is designated *CYP3A5\*3* [16]. Recently, we reported that the maintenance oral dosage of tacrolimus was 40-fold lower than the dose before co-administration with ITCZ in allogeneic HSCT recipients with *CYP3A5\*3/\*3* alleles [17]. To our knowledge, thus far, no study has systematically evaluated the interactions between orally administered CNIs and ITCZ or determined whether the magnitude of interaction is different among patients harboring genetic polymorphisms in *CYP3A5*. Therefore, the aim of the study reported here was to investigate the interactions between orally administered ITCZ and tacrolimus or cyclosporine in Japanese allogeneic HSCT recipients.

## Material and methods

### Patients and protocols

A single-institute, prospective pilot study was conducted to evaluate both the efficacy and safety of an oral solution of ITCZ and its interactions with tacrolimus or cyclosporine in allogeneic HSCT recipients.

The patient eligibility criteria for the study were as follows: (1) HSCT recipient treated using identical immunosuppressive regimens that included tacrolimus (Prograf®) and cyclosporine (Neoral®); (2) age of >16 years; (3) no hypersensitivity to ITCZ; (4) no liver dysfunction [aspartate aminotransferase (AST) or alanine aminotransferase (ALT) level of <3-fold the upper normal range and total bilirubin level of <2.0 mg/dL]; (5) no renal dysfunction (serum creatinine level of <2.0 mg/dL or creatinine clearance of >30 mL/min); (6) nonsmoker; (7) no consumption of drugs or food affecting CYP3A and P-glycoprotein levels.

After HSCT, continuous infusion of 0.03 mg/kg/day tacrolimus or 2.0–3.0 mg/kg/day cyclosporine (from day –1 before transplantation) was administered to prevent acute graft-versus-host disease (aGVHD). On days 20–30 after transplantation, patients were switched from continuous

CNI infusion to 0.06 mg/kg/day orally administered tacrolimus or 6–12 mg/kg/day orally administered cyclosporine in equal doses every 12 h at 0800 and 2000 hours. After the initiation of 200 mg ITCZ twice daily (1000 and 2200) for the first 2 days as a loading dose, a 200 mg/day ITCZ oral solution was administered at 0600 hours on days 30–100 after transplantation. The target trough concentrations of tacrolimus and cyclosporine were 5–15 ng/mL and 150–300 ng/mL, respectively.

Treatment was terminated if participants met any one of the following criteria: (1) fungal infection; (2) no resolution of fever (temperature >38 °C for 4–7 days) by a broad-spectrum antibacterial agent during neutropenia; (3) drug-related toxicity; (4) AST or ALT level of >5-fold the upper normal range; (5) total bilirubin level of >3 mg/dL; (6) serum creatinine level of >2.0 mg/dL or creatinine clearance of <30 mL/min; (7) aggravation of a hematological disease or a complication; (8) grade 3/4 aGVHD.

A total of 16 Japanese HSCT patients (12 men, 4 women) who received grafts between October 2009 and August 2011 were enrolled in the study. The study protocol was approved by the Ethics Committee of Akita University Graduate School of Medicine, and all patients gave written informed consent.

### Sample collection and analytical methods

Before the co-administration of ITCZ and daily for 1 week thereafter, we collected venous blood samples just before ( $C_{0h}$ ) and 2 h ( $C_{2h}$ ) after tacrolimus or cyclosporine administration (0800 hours) to determine the blood concentrations of tacrolimus and cyclosporine and the plasma concentrations of ITCZ and hydroxyitraconazole. Tacrolimus and cyclosporine blood concentrations were determined using chemiluminescence magnetic microparticle immunoassay (CMIA) on the Architect-i1000® system (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions. The limit of quantitation of tacrolimus and cyclosporine for the Architect-i1000® instrument is stated to be 0.5 ng/mL [18] and 20 ng/mL [19], respectively, and their coefficients of variation for this assay were <10 % in the concentration range 2–8 ng/mL [18] and <15 % in the concentration range 87.5–916 ng/mL [19], respectively. Plasma concentrations of ITCZ and hydroxyitraconazole were monitored using high-performance liquid chromatography (HPLC) [20]. The limit of quantitation of this HPLC system for ITCZ and hydroxyitraconazole is stated to be 0.5 ng/mL [20], and the coefficient of variation for both substances in this assay is <13 % in the concentration range 5–2,500 ng/mL [20].

### Genotyping

DNA was extracted from a peripheral blood sample using the QIAamp Blood kit (Qiagen, Hilden, Germany) and

stored at  $-80^{\circ}\text{C}$  until analysis. For genotyping, the *CYP3A5*\*3 allele was detected using a PCR–restriction fragment length polymorphism (RFLP) method [21]. The results obtained from PCR-RFLP were confirmed using a fully automated SNP detection system (prototype i-densy<sup>TM</sup>; ARKRAY, Kyoto, Japan).

### Statistical analyses

The Kolmogorov–Smirnov test was used to assess distribution. The doses of tacrolimus or cyclosporine were expressed as the mean  $\pm$  standard deviation (SD). The clinical characteristics of patients with HSCT, dose-adjusted blood concentrations, and changes in these parameters were expressed as medians (quartiles 1–3). The chi-square test was used to examine differences in categorical data, except when expected number of cells was  $<5$ , in which case the Fisher's exact test was used. Statistically significant differences between two populations were assessed using the Student's *t* test if the population data conformed to a normal distribution; otherwise, the Mann–Whitney *U* test was applied. The Wilcoxon paired signed rank test was used to determine the difference in continuous values within each patient. Spearman's rank

correlation coefficient test was used to assess the correlation between the dose-adjusted blood concentration of tacrolimus or cyclosporine and the plasma concentration of ITCZ. These results were expressed as a determination coefficient ( $r^2$ ). A *P* value of  $<0.05$  was considered to be statistically significant. Statistical analyses were performed using SPSS ver. 20.0 software for Windows (SPSS IBM Japan, Tokyo, Japan).

### Results

Patient characteristics are listed in Table 1. The genotype frequencies of the *CYP3A5* polymorphisms were almost equal in the tacrolimus and cyclosporine groups.

No patients were removed from this study because of the previously mentioned criteria, which included breakthrough fungal infections, severe aGVHD [22], or severe adverse events (Table 2).

The steady-state median (quartiles 1–3) plasma concentrations of ITCZ in the tacrolimus and cyclosporine groups were 1,005 (768–1,403) and 1,357 (682–2,122) ng/mL, respectively, and those of hydroxyitraconazole were 1,023 (872–1,674) and 1,367 (1,056–1,904) ng/mL, respectively.

**Table 1** Clinical characteristics of hematopoietic stem cell transplant recipients taking tacrolimus or cyclosporine

Clinical characteristics	Calcineurin inhibitor		<i>P</i>
	Tacrolimus	Cyclosporine	
Number of patients	8	8	–
Women/men	3/5	1/7	0.2850
Age (years)	53 (33–61)	47 (28–57)	0.5054
Body weight (kg)	60.5 (54.7–66.1)	57.0 (50.4–61.2)	0.5054
<i>CYP3A5</i> genotype			0.8560
*1/*1	1	1	
*1/*3	3	2	
*3/*3	4	5	
Diagnosis			0.8425
AML/MDS	4	3	
ALL/CML-lymphoid crisis	2	3	
NHL	2	2	
Graft source			0.0286
Bone marrow of unrelated donor	4	5	
PBSC of sibling donor	0	3	
Umbilical cord blood	4	0	
HLA locus matching			0.0003
4/6	2	0	
5/6	6	0	
6/6	0	8	
Conditioning			0.5000
Reduced intensity	5	4	
Myeloablative	3	4	

*CYP*, Cytochrome P450; *AML*, acute myeloid leukemia; *MDS*, myelodysplastic syndrome; *ALL*, acute lymphoid leukemia; *CML*, chronic myeloid leukemia; *NHL*, non-Hodgkin lymphoma; *PBSC*, peripheral blood stem cells; *HLA*, human leukocyte antigen

Values are expressed as the number or as the median with the range in parenthesis

**Table 2** Acute graft-versus-host disease and fungal infection during the study

Calcineurin inhibitor	Tacrolimus (n=8)	Cyclosporine (n=8)	P
Acute GVHD			0.3114
Grade 0	1	3	
Grade 1	1	2	
Grade 2	6	3	
Grade 3/4	0	0	
PSL for acute GVHD	4	4	0.5000
Fungal infection	0	0	–

GVHD, Graft-versus-host disease; PSL, systemic administration of prednisolone

The median (quartiles 1–3) hydroxyitraconazole/ITCZ concentration ratios in the tacrolimus and cyclosporine groups were 1.23 (0.81–1.67) and 1.20 (1.08–1.38), respectively. ITCZ co-administration did not result in any serious renal or hepatic dysfunction, and no laboratory abnormalities were observed (Table 3).

**Table 3** Itraconazole and hydroxyitraconazole concentrations and laboratory data before and after co-administration of itraconazole

Traconazole and hydroxyitraconazole concentrations <sup>a</sup> and laboratory data	Calcineurin inhibitor		P <sup>b</sup>
	Tacrolimus (n=8)	Cyclosporine (n=8)	
Concentrations			
ITCZ (ng/mL)	1005 (768–1,403)	1357 (682–2,122)	0.6454
OH-ITCZ (ng/mL)	1023 (872–1,674)	1367 (1,056–1,904)	0.5737
OH-ITCZ:ITCZ Ratio	1.23 (0.81–1.67)	1.20 (1.08–1.38)	0.9591
Laboratory data			
Aspartate transaminase			
Before	35 (22–40)	27 (17–32)	0.3282
Day 7	24 (21–27)	19 (16–22)	0.1949
P <sup>c</sup>	0.0422	0.0357	
Alanine transaminase			
Before	48 (27–80)	29 (16–65)	0.2786
Day 7	31 (25–53)	19 (14–25)	0.0830
P <sup>c</sup>	0.0300	0.0173	
Serum albumin			
Before	3.9 (3.6–4.3)	3.8 (3.7–4.0)	0.7209
Day 7	4.0 (3.7–4.3)	3.8 (3.8–4.0)	0.6454
P <sup>c</sup>	0.4959	0.8924	
Total bilirubin			
Before	0.6 (0.3–1.0)	0.6 (0.4–0.6)	0.7984
Day 7	0.6 (0.5–1.1)	0.7 (0.6–1.0)	0.4418
P <sup>c</sup>	0.1308	0.0174	
Serum creatinine			
Before	0.62 (0.55–0.82)	0.69 (0.60–0.81)	0.6454
Day 7	0.77 (0.60–0.92)	0.73 (0.55–0.85)	0.9591
P <sup>c</sup>	0.3621	0.4990	

ITCZ, Itraconazole; OH-ITCZ, hydroxyitraconazole

Values are expressed as the number or as the median (quartiles 1–3)

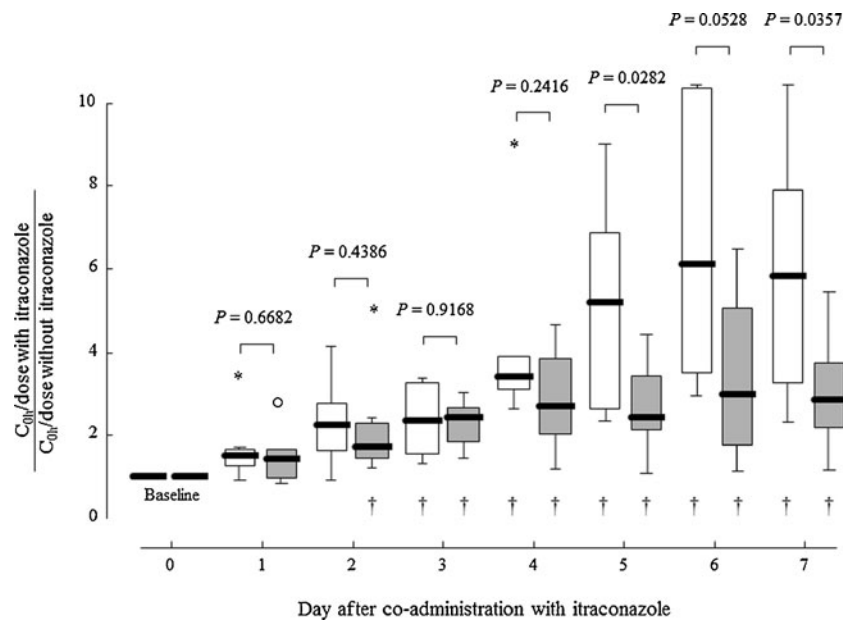
<sup>a</sup>Plasma concentrations of itraconazole and hydroxyitraconazole were calculated from mean values from day 3 to day 7 after ITCZ co-administration

<sup>b</sup>P tacrolimus vs. cyclosporine

<sup>c</sup>P before vs. day 7

The ratio of dose-adjusted C<sub>0h</sub> and C<sub>2h</sub> values with or without ITCZ is plotted against the number of days after co-administration with ITCZ for the tacrolimus and cyclosporine groups and shown in Figs. 1 and 2, respectively. ITCZ treatment significantly increased the dose-adjusted C<sub>0h</sub> of tacrolimus and cyclosporine from day 3 after the start of ITCZ co-administration (Fig. 1). The median (quartiles 1–3) dose-adjusted C<sub>0h</sub> of tacrolimus and cyclosporine on day 7 increased by 5.6- (3.1–7.5) or 2.7- (2.1–3.6) fold, respectively, compared with the values before the initiation of ITCZ. A significant inter-group difference was observed in the dose-adjusted C<sub>0h</sub> after ITCZ treatment (P=0.0357), indicating that the drug interaction with ITCZ was greater than that for tacrolimus.

Moreover, ITCZ treatment significantly increased the dose-adjusted C<sub>2h</sub> of tacrolimus from day 4 after the start of ITCZ co-administration, whereas no significant difference was observed in the dose-adjusted C<sub>2h</sub> of cyclosporine up to 7 days after the start of ITCZ co-administration (Fig. 2). The median (quartiles 1–3)

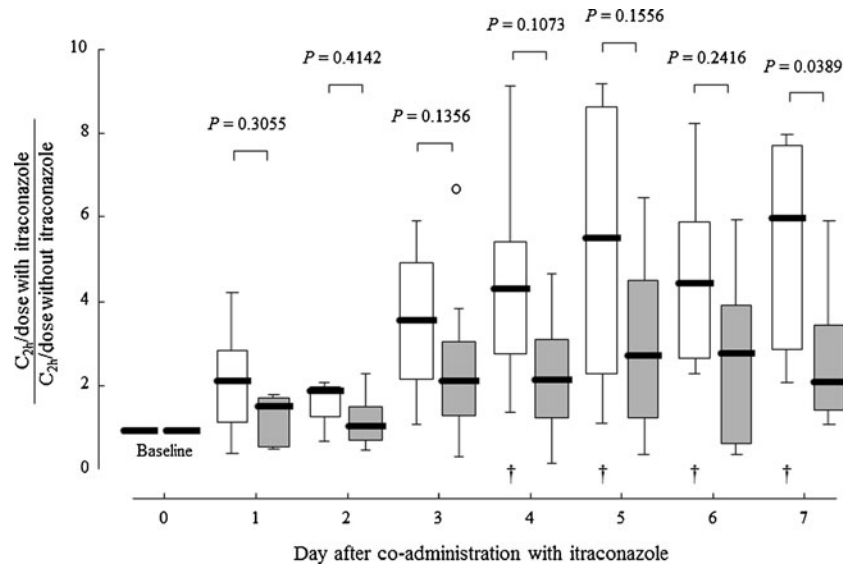


**Fig. 1** The ratio of dose-adjusted blood trough concentration ( $C_{0h}$ ) with or without itraconazole (ITCZ) is plotted as a function of the number of days after the co-administration of ITCZ with tacrolimus (open column) and cyclosporine (closed column) in hematopoietic stem cell transplant recipients. This graphical analysis was carried out using the SPSS (SPSS, Chicago, IL) box and whiskers plot. The box spans data between the two quartiles (interquartile range, IQR),

with the median represented as a bold horizontal line. The ends of the whiskers (vertical lines) represent the smallest and largest values that are not outliers. Outliers (circles) are values between 1.5 and 3 IQRs from the end of a box. Values of >3 IQRs from the end of a box are defined as extreme (asterisk). † $P < 0.05$  indicates that a significant difference was observed before and after ITCZ treatment

dose-adjusted  $C_{2h}$  of tacrolimus and cyclosporine on day 7 after the start of ITCZ co-administration increased by 6.0- (2.9–7.7) or 2.1-fold (1.5–3.5), respectively, compared with the median before the initiation of ITCZ

therapy. Similar to the  $C_{0h}$  results, the tacrolimus and cyclosporine groups were significantly different in terms of the dose-adjusted  $C_{2h}$  after the start of ITCZ co-administration ( $P=0.0389$ ).



**Fig. 2** The ratio of dose-adjusted blood concentration ( $C_{2h}$ ) with or without ITCZ is plotted as a function of the number of days after the co-administration of ITCZ for tacrolimus (open column) and cyclosporine (closed column) in hematopoietic stem cell transplant recipients. Graphical analysis was carried out using the SPSS box and whiskers plot. The box spans data between the two quartiles (IQRs),

with the median represented as a bold horizontal line. The ends of the whiskers (vertical lines) represent the smallest and largest values, respectively, that are not outliers. Outliers (circles) are values between 1.5 and 3 IQRs from the end of a box. † $P < 0.05$  indicates that a significant difference was observed before and after ITCZ treatment

The reduction in the single dosage of tacrolimus and cyclosporine after the start of ITCZ co-administration is shown in Fig. 3. The mean single dosage of tacrolimus and cyclosporine was decreased to 33.7 % and 66.5 %, respectively, of that before ITCZ co-administration. The reduction rate and the time for the single dosage of tacrolimus were significantly greater and faster, respectively, than that for cyclosporine.

The correlation between the  $C_{0h}$  of tacrolimus or cyclosporine and the plasma concentrations of ITCZ from days 3 to 7 is shown in Fig. 4. The correlation between the  $C_{0h}$  of cyclosporine and plasma ITCZ concentrations was high ( $r^2=0.4141$ ,  $P<0.0002$ ); however, no correlation was found between tacrolimus and ITCZ.

The changes in the dose-adjusted  $C_{0h}$  of tacrolimus and cyclosporine before and on day 7 after the start of ITCZ co-administration in individual patients and their CYP3A5 genotypes are shown in Fig. 5. The dose-adjusted  $C_{0h}$  of tacrolimus in the single patient with the CYP3A5\*1/\*1 genotype did not change following ITCZ co-administration; however, the concentration of tacrolimus in the other patients with the CYP3A5\*1/\*3 and \*3/\*3 genotypes did significantly increase after the start of ITCZ co-administration. In contrast, this CYP3A5 genotype-associated tendency was not observed as a clear trend in patients receiving cyclosporine.

## Discussion

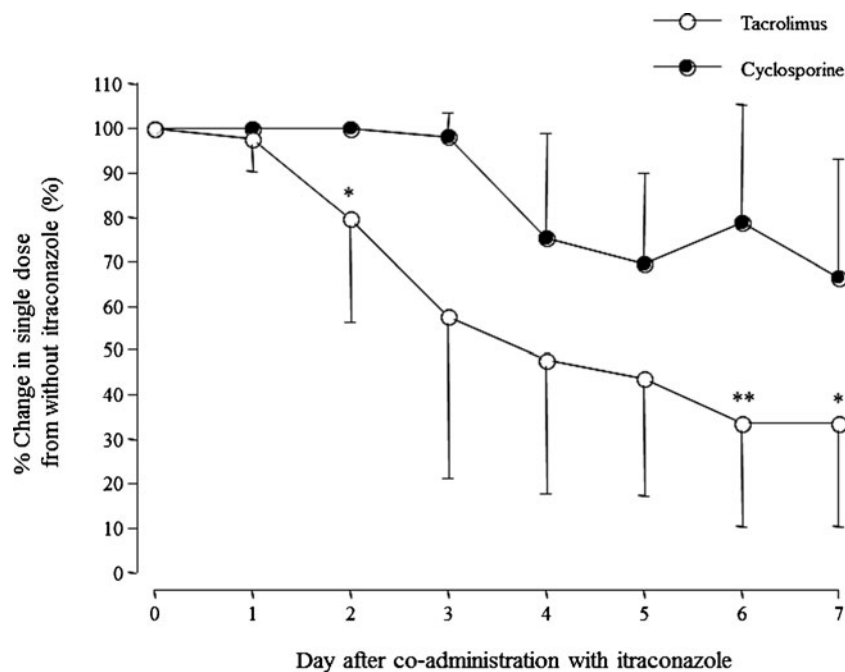
The present study is the first report, to our knowledge, of a systematic evaluation of the interactions between orally

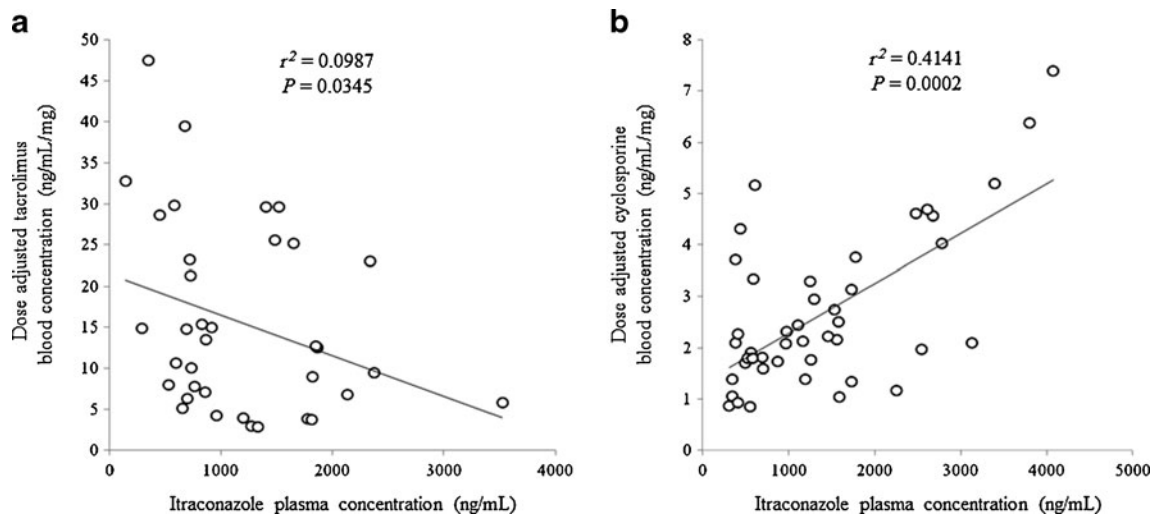
administered ITCZ and CNIs and an investigation of possible variations in the magnitude of drug interactions among patients with different CYP3A5 genetic polymorphisms.

In an in vitro study using human liver microsomes from subjects harboring the CYP3A5\*3/\*3 genotype, Yamazaki et al. reported that the inhibition constant ( $K_i$ ) of ITCZ for CYP3A5\*3/\*3 is approximately half that of the CYP3A5\*1/\*3 genotype [15]. The authors of this study expected that the increased area under the concentration–time curve (AUC) of CYP3A5 substrate resulting from ITCZ co-administration in patients with the CYP3A5\*1/\*1 allele would be the lowest of the three CYP3A5 genotype groups [15]. In our study, the dose-adjusted  $C_{0h}$  of tacrolimus in the one patient with the CYP3A5\*1/\*1 genotype was not changed by ITCZ co-administration; however, the concentration of tacrolimus in the other patients with the CYP3A5\*1/\*3 and \*3/\*3 alleles significantly increased after the start of ITCZ co-administration.

Taken together, these findings suggest that in homozygous expressors of CYP3A5\*1/\*1, the interaction is minimal, presumably because the CYP3A5 pathway is fully capable of compensating for the lack of drug metabolism of the inhibited CYP3A4 pathway. In heterozygous expressors of CYP3A5\*1/\*3, the interaction manifests more than that of the homozygous (\*1/\*1) phenotype because there are fewer active CYP3A5 molecules to compensate for the effects of the inhibited CYP3A4 pathway. Our present findings support the results reported by Yamazaki et al. [15]. In contrast, it appears that the cyclosporine and ITCZ interaction is not as influenced by or is sensitive to CYP3A5 polymorphisms. The authors of several studies also report that CYP3A5 polymorphisms significantly influenced treatment

**Fig. 3** The percentage change in the single dosage of tacrolimus and cyclosporine from day 0 without itraconazole. Open circles Tacrolimus, closed circles cyclosporine. \* $P<0.05$  compared with the cyclosporine group





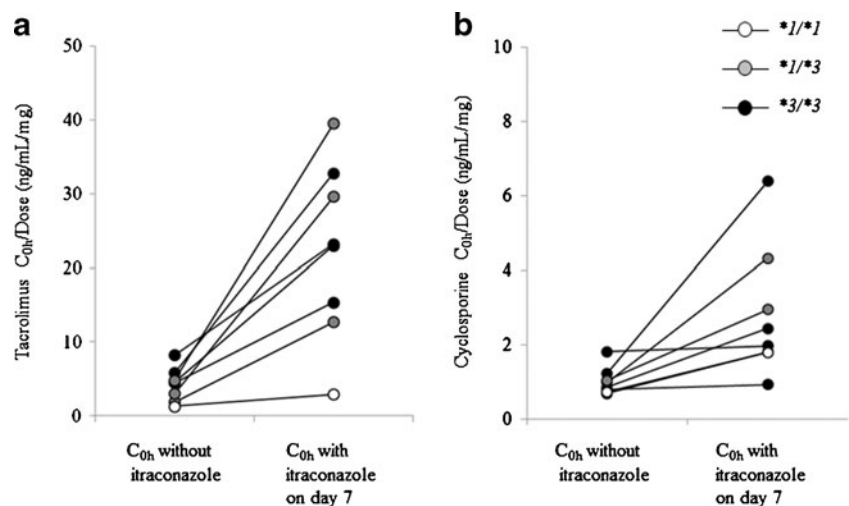
**Fig. 4** Correlation between the  $C_{0h}$  of tacrolimus (a) or cyclosporine (b) and the plasma concentration of itraconazole

with tacrolimus, but not with cyclosporine [23, 24]. Leather et al. reported that intravenously administered ITCZ increased the mean serum concentration of tacrolimus and cyclosporine by 83 and 80 %, respectively, in allogeneic HSCT recipients [25]. This result shows that the interactions between ITCZ and intravenously administered CNIs involve hepatic CYP3A and that the magnitude of the drug interactions between intravenous tacrolimus or cyclosporine and ITCZ are very similar [25]. However, in our study, the magnitude of the interaction between orally administered tacrolimus and ITCZ was significantly greater than that between orally administered cyclosporine and ITCZ. Therefore, intestinal CYP3A5 might play an important role in the metabolism of tacrolimus [26]. If tacrolimus is selected as an immunosuppressive agent for allogeneic HSCT patients, the prospective analysis of *CYP3A5* polymorphism may be important to ensure safe and reliable immunosuppressive therapy with tacrolimus.

The ITCZ metabolites hydroxyitraconazole, ketoitraconazole, and *N*-desalkyl-itraconazole also inhibit CYP3A4 in vitro, and they may contribute to the overall inhibition of CYP3A4 in vivo if they circulate at sufficiently high concentrations [10, 27]. Although only hydroxyitraconazole was measured in our study, this metabolite might influence the metabolism of tacrolimus via CYP3A4. Moreover, tacrolimus inhibits CYP3A4 and CYP3A5, whereas cyclosporine only inhibits CYP3A4 [28]. Because the inhibition of CYP by tacrolimus is much stronger than that of cyclosporine, the co-administration of CNIs and ITCZ in allogeneic HSCT patients may be easier to manage using cyclosporine rather than tacrolimus.

CNIs are also a substrate of P-glycoprotein, and ITCZ is a known potent inhibitor of P-glycoprotein activity, both in vitro and in vivo [29, 30]. P-glycoprotein is a membrane efflux transporter that is normally expressed in human tissues, such as the small intestine, the biliary canalicular front of hepatocytes, and renal proximal tubules [31]. Orally

**Fig. 5** Comparison of the  $C_{0h}$  of tacrolimus (a) and cyclosporine (b) according to the presence or absence of co-administered itraconazole. Open circles *CYP3A5*\*1/\*1, gray circles *CYP3A5*\*1/\*3, closed circles *CYP3A5*\*3/\*3. CYP Cytochrome P450



administered ITCZ may also increase the blood concentrations of CNIs by inhibiting P-glycoprotein activity in the small intestine or on the biliary canalicular front of hepatocytes.

No laboratory abnormalities were observed in the recipients of ITCZ co-administration. Although allogeneic HSCT patients received a loading dose of ITCZ intravenously for 2 days followed by oral treatment with 200 mg of ITCZ, the dose-adjusted  $C_{0h}$  values of tacrolimus and cyclosporine were significantly increased from day 3 and then stabilized on day 5 after the start of ITCZ co-administration. Thus, the interactions between the CNIs and ITCZ were observed 3 days after replacement of the oral solution. Because sufficient time may be required for the appearance of drug interactions, the CNI  $C_{0h}$  should be monitored daily for at least 5 days after the start of ITCZ co-administration to prevent the occurrence of adverse events. The reduction rate and the time for the single dosage of tacrolimus were significantly greater and faster than that of cyclosporine. These findings also suggest that the magnitude of the drug interaction between orally administered tacrolimus and ITCZ was significantly greater than that between cyclosporine and ITCZ. Further, the therapeutic window of tacrolimus is very narrow, and its target concentration might be too low for it to be adjusted compared to cyclosporine.

It is important to note that this study was performed with a small number of patients. Therefore, our results must be interpreted within the context of this limitation, and further examination with a larger sample size to corroborate our current findings is necessary.

**Conflict of interest** None.

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