Fluconazole (FCZ), a triazole antifungal agent, is commonly administered as prophylaxis for fungal infections in patients receiving hematopoietic stem cell transplantation (HSCT). In the “Guidelines for Preventing Opportunistic Infections among HSCT recipients: Recommendations of the Centers for Disease Control, the Infectious Diseases Society of America, and the American Society of Blood and Marrow Transplantation,” FCZ should be administered from the day of HSCT (day 0) until engraftment. FCZ is highly effective against Candida species, but ineffective against mold pathogens such as Aspergillus species that are being increasingly described in HSCT recipients. Thus, in a case of breakthrough fungal infections, antifungal agents other than FCZ will be administered as empiric therapy (e.g., voriconazole (VCZ), itraconazole, micafungin, and amphotericin B etc.). In particular, VCZ is a novel triazole antifungal agent with potent activity against a broad spectrum of clinically significant mold pathogens, including Aspergillus, Cryptococcus, and Candida species.

These triazole antifungal agents have interaction against calcineurin inhibitors including tacrolimus (FK506) and cyclosporin A (CyA). Calcineurin inhibitors are routinely administered as prophylaxis for graft versus host disease (GVHD) in patients receiving allogeneic HSCT (allo-HSCT). Triazole antifungal agents are metabolized by the cytochrome P450 (CYP) enzyme system, CYP2C9, CYP2C19, and CYP3A4 isozymes, whereas the calcineurin inhibitors are primarily metabolized by CYP3A4. Therefore, the triazole antifungal agents competitively inhibit metabolism of the calcineurin inhibitors. Consequently, the concentrations of FK506 and CyA were increased when triazole antifungal agents were concomitantly administered. In vitro, the IC_{50} value of VCZ against CYP3A4 activity was comparable with that of FCZ. However, in vivo, Kami M., et al. described that VCZ has more intensive interaction than FCZ. Thus, it is expected that the switch from FCZ to VCZ will change the concentration of FK506. Therefore, a dose adjustment of FK506 is necessary to avoid side effects such as nephrotoxicity, but the precise dose is unknown.

Therefore, the purpose of this study was to assess the impact by switching co-administered triazole antifungal agent from fluconazole (FCZ) to voriconazole (VCZ) on the blood concentration of tacrolimus (FK506) in patients receiving allogeneic hematopoietic stem cell transplantation. We performed a retrospective study presented as case reports. The blood concentration of FK506 was increased after the switch from FCZ to VCZ, resulting in increase of the concentration/dose ratio (C/D) of FK506. Thus, the mean C/D ratios of FK506 with oral administration was surprisingly increased over 4.5-fold after the switch. Therefore, it was necessary to reduce the FK506 dose when co-administered FCZ is switched to VCZ. We should be careful when interpreting the results of these case reports; however, in some patients, it is recommended that the dose of FK506 be reduced to one-fifth after the switch.

**Key words** voriconazole; tacrolimus; transplantation; concentration; interaction

**MATERIALS AND METHODS**

**Study Design** This study was retrospective and it is consequently presented as case reports because of the lack of patient numbers. This study was approved by the Institutional Review Board of Kagawa University Hospital.

**Subjects** Twenty-five Japanese patients underwent allo-HSCT in this hospital between April 2005 and March 2006. In three subjects, antifungal agent co-administered with FK506 was switched from FCZ to VCZ. The characteristics of these subjects are shown in Table 1. They had no medicine other than the antifungal agents interacting against FK506. Twenty-two other patients were excluded from this study as the empiric therapy was mainly unnecessary, that is, the prophylactic administration of FCZ was sufficient and an antifungal agent other than FCZ was initially administered as prophylaxis.

**Regimen** The following is the basic regimen of allo-HSCT in this hospital. FCZ (Diflucan®) is orally administered as prophylaxis for fungal infections from day −14 until before or after day 100. If a patient has severe vomiting from regimen-related toxicity of chemotherapy, FCZ is intravenously administered. In this report, fosfluconazole (Prodif®, a pro-drug of FCZ and an injection medicine, was administered venously administered. In this report, fosfluconazole (Prodif®, a pro-drug of FCZ and an injection medicine, was administered venously administered. In this report, fosfluconazole (Prodif®, a pro-drug of FCZ and an injection medicine, was administered.
considered FCZ for convenience. FCZ is switched to VCZ (Vfend®) as an alternative empiric therapy in a case of breakthrough fungal infections. On the other hand, FK506 (Prograf®) is intravenously administered as prophylaxis for GVHD using a continuous infusion (0.01 mg/kg/d) from the day before allo-HSCT (day −1) until engraftment. If a patient is making satisfactory progress after allo-HSCT, FK506 is orally administered. The dose of FK506 is empirically adjusted to maintain the optimum concentration (10—15 ng/ml) if necessary.

FK506 Assay The concentrations of FK506 were quantified using the IMx Tacrolimus II assay (Abbot Diagnostics, Abbott Park, IL, U.S.A.) with microparticle enzyme immunoassay technology. The limit range of quantification was from 1.5—30 ng/ml. Blood samples were routinely obtained three times a week at trough or steady-state level with oral or intravenous administration of FK506, respectively.

Assessment The blood concentrations of FK506 were normalized for the daily dose of FK506 (ng/ml per mg/kg)1,14,20; that is, the concentration/dose (C/D) ratio was used to assess the impact by switching co-administered triazole antifungal agent from FCZ to VCZ on the blood concentration of FK506. The following values were excluded from this assessment as FK506 was discontinued for high concentration, because the daily dose of FK506 was unknown.

Statistical Analysis Values are indicated as the mean± standard error (S.E.). Comparison between the mean C/D ratios of FK506 at the time of co-administration with FCZ and VCZ was analyzed using Welch’s t-test after that all data were tested for normal distribution with the Kolmogorov–Smirnov goodness of fit test. All p-values were two-tailed and p<0.05 was considered significant.

RESULTS

The changes of C/D ratios of FK506 with oral administration at the time of co-administration with FCZ and VCZ were analyzed using Welch’s t-test after that all data were tested for normal distribution with the Kolmogorov–Smirnov goodness of fit test. All p-values were two-tailed and p<0.05 was considered significant.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Disease/Status</th>
<th>Type of transplantation</th>
<th>GVHD prophylaxis</th>
<th>CCr (ml/min)</th>
<th>T.Bil (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>46</td>
<td>Female</td>
<td>46</td>
<td>AML/NCR</td>
<td>CBT</td>
<td>RIST</td>
<td>FK506</td>
<td>95 (48—176)</td>
</tr>
<tr>
<td>No. 2</td>
<td>63</td>
<td>Male</td>
<td>62</td>
<td>MDS/CR</td>
<td>Unrelated BMT</td>
<td>RIST</td>
<td>FK506+SMTX</td>
<td>70 (40—88)</td>
</tr>
<tr>
<td>No. 3</td>
<td>50</td>
<td>Female</td>
<td>52</td>
<td>AML/NCR</td>
<td>CBT</td>
<td>RIST</td>
<td>FK506</td>
<td>158 (38—230)</td>
</tr>
</tbody>
</table>

GVHD: graft versus host disease, CCr: creatinine clearance, T.Bil: total bilirubin value, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, NCR: non-complete remission, CR: complete remission, CBT: cord blood transplantation, BMT: bone marrow transplantation, RIST: reduced intensity stem cell transplantation, FK506: tacrolimus, SMX: short-term methotrexate. a) CCr was calculated with the Cockcroft–Gault equation using the serum creatinine value. b) The values are indicated throughout the investigation period in each patient, respectively.

Fig. 1. Shift of the C/D Ratio of FK506 with Oral Administration in Subject No. 1 (A) and No. 2 (B)

Close circles and open circles show the C/D ratios of FK506 at the time of co-administration with FCZ and VCZ, respectively. FCZ: fluconazole, VCZ: voriconazole, FK506: tacrolimus, p.o.: per os administration, i.v.: intravenous administration.
ng·ml⁻¹/mg·kg⁻¹, respectively. Thus, the shift of the C/D ratio was 4.9-fold increase. In subject No. 2, the mean C/D ratios of FK506 with VCZ (400 mg, i.v.; 890±164) and VCZ (300—200 mg, p.o.; 1249±135) were shifted to 3.2-fold and 4.5-fold increase, respectively, compared to that with FCZ (200 mg, p.o.; 278±21). The mean C/D ratios of FK506 with intravenous administration at the time of co-administration with FCZ (200 mg, p.o.) in subject No. 1 and No. 2 were 1237±80 and 1522±91, respectively (data not shown).

The subject No. 3 also showed the increase of the C/D ratios of FK506 with intravenous administration after the switch of FCZ to VCZ (Fig. 2A). The blood total bilirubin value (T.Bil) representatively substituted as liver function parameter for convenience was increased in this subject (Table 1). The C/D ratios of FK506 was positively correlated with T.Bil ($r^2=0.782, p=0.0003, \text{Fig. 2B}$), whereas not with creatinine clearance (CCr) as a renal function parameter for convenience (data not shown). In other two subjects, there was no correlation between C/D ratios of FK506 and CCr, or T.Bil in each group throughout the investigation period (data not shown).

**DISCUSSION**

FCZ is switched to VCZ in a case of breakthrough fungal infections. Both triazole antifungal agents are well known to interact with FK506 at CYP3A4, however it is little known how switching from FCZ to VCZ impacts the blood concentration of FK506. This study shows that the mean C/D ratio of FK506 was surprisingly increased over 4.5-fold in the patients receiving allo-HSCT after co-administrated agent was switched to VCZ from FCZ (Table 2). Therefore, the dose of FK506 may be necessary to be reduced to one-fifth after the switch.

Although, in vitro, the IC₅₀ value of VCZ against CYP3A4 activity was comparable with that of FCZ, the blood concentration of FK506 was increased after the switch from FCZ to VCZ, resulting in increase of the C/D ratio of FK506. One possible reason for the potent influence of VCZ found in vivo is that the concentration of VCZ at liver and intestine might be higher than that of FCZ because of the difference of dosage. FCZ and VCZ competitively inhibit CYP2C9, CYP2C19, and CYP3A4. The pre-administration of FCZ might increase the VCZ concentration as well as inhibit FK506 metabolism as carry-over effect. But in subject No. 2 the increase of C/D ratio of FK506 was shown even if the washout period was established when switching to VCZ from FCZ. The manufacturer recommends that FK506 dose be reduced to one-third of the original dose when initiating therapy with VCZ in patients already receiving FK506. But more reduction of FK506 dose was needed when the same dose of VCZ was administered following FCZ.

CYP3A4 is the most abundant isozyme of CYP, accounting for nearly 30% of the total CYP content in the human liver and as much as 70% in the intestinal wall. Therefore, it is expected that the oral administration of calcineurin inhibitors has a more intensive impact of interaction than intravenous administration. In two reviews, there was an impact of interaction between triazole antifungal agents and calcineurin inhibitors when both medicines were orally administered. In our result (Fig. 1), the C/D ratio of FK506 with oral administration was increased by switching from FCZ to VCZ. On the other hand, the contradictory results was reported when both medicines were intravenously ad-
ministered; an impact of interaction by Leather H., et al.3) and no impact by Osowski C. L., et al.4) In our result (Fig. 2A), the C/D ratio of FK506 with intravenous administration was also increased by switching from FCZ to VCZ. However, there was a significant correlation between the C/D ratio and T.Bil regardless of the impact of antifungal agents (r²=0.782, p=0.0003, Fig. 2B). Therefore, the changes of C/D ratio of FK506 shown in subject No. 3 may be due to liver dysfunction.

There are several clinical reports regarding the interaction with VCZ and FK506 only in which the initiation of VCZ administration increased the concentration of FK506 in patients already receiving FK506.7,8,12—16 This is a novel report regarding the intensity of interaction with VCZ and FK506 by switching from FCZ in patients receiving allo-HSCT. In the clinical situation mentioned above, we will frequently encounter this situation, stressing the significance of this study. As clinical significance, day 100 is the median day for the development of fungal infections, during which most patients underwent allo-HSCT are followed as outpatients.3) In outpatients, it is particularly necessary to reduce the dose of FK506, as well as monitor the blood concentration of FK506 after the switch from FCZ to VCZ.

In the future, the impact of interaction between triazole antifungal agents and calcineurin inhibitors should be investigated in a large number of patients receiving allo-HSCT when switching antifungal agents. We should be careful when interpreting the results of these case reports; however, the following conclusion can be reached: 1) It is necessary to reduce the FK506 dose when co-administered FCZ is switched to VCZ; 2) in some patients, it is recommended that the dose of FK506 be reduced to one-fifth after the switch.

REFERENCES AND NOTES


