Analysis of the Blood Level of Micafungin Involving Patients with Hematological Diseases: New Findings Regarding Combination Therapy with Tacrolimus

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In 29 patients (40 samples) with hematological diseases who had been treated with a candin antifungal agent, micafungin (MCFG), we measured the blood level of MCFG by high-performance liquid chromatography (HPLC). There was a correlation between the dose and the blood level of MCFG (r=0.729, p<0.001). In addition, there was a correlation between the total bilirubin level and the C/D value (r=0.458, p<0.01), which was calculated by dividing the blood level of MCFG by the dose, although there was no correlation between creatinine clearance and the C/D value. These findings suggest that the dose of MCFG must be regulated in patients with biliary stasis-type liver hypofunction. In addition, there was no significant difference in the blood level of MCFG between the group in which tacrolimus (FK506) was combined with MCFG and the group in which MCFG alone was administered. These results suggest that there are no changes in the blood level of MCFG even when MCFG is combined with FK506.

Key words micafungin; blood level; tacrolimus; hematological disease; Aspergillus

The action mechanism of a new candin antifungal agent, micafungin (MCFG), which was developed as the sixth antifungal agent in Japan, differs from that of conventional antifungal agents, and this agent may be clinically useful. MCFG has a broad spectrum against Candida and Aspergillus in vitro by inhibiting the biosynthesis of the main constituent comprising the fungal cellular wall, 1,3-β-D glucan.1,2 As this agent shows favorable lung transfer,3 it may be useful for preventing and treating pulmonary mycosis.

We previously reported the efficacy of this agent and the incidence of side effects in 17 patients with hematological diseases to whom MCFG was administered to treat/prevent pulmonary aspergillosis.4 Our study suggested that 150 mg or more of MCFG is required to treat/prevent pulmonary aspergillosis; however, the necessity of adjusting the dose in patients with liver/kidney hypofunction and the presence or absence of interactions with other agents remain to be clarified. As an immunosuppressive agent, tacrolimus (FK-506), is administered to patients after hematopoietic stem cells transplantation from non-relatives for a long period, it should be confirmed whether interactions related to combination therapy with MCFG appear.

In this study, we measured the blood level of MCFG in 29 patients with hematological diseases, and analyzed the data. We report findings regarding administration to patients with liver/kidney hypofunction and combination therapy with FK-506.

MATERIALS AND METHODS

Subjects The subjects were 29 patients who were admitted to the First Department of Internal Medicine (Department of Hematology) in Nagano Red Cross Hospital between February 2003 and October 2004. Written informed consent regarding the measurement of the blood MCFG level was obtained from the patients or their families. As a rule, blood was collected 3 d or more after the start of administration, when MCFG reached a steady state, to obtain a trough value. If necessary, blood was collected in accordance with the general condition.

Informed Consent In obtaining informed consent from the patients or their families, we paid attention to protect the patients’ rights according to the Helsinki declaration. In addition, it was explained that blood samples were not used for purposes other than this study. The Ethics Committee in our hospital approved this study.

Preparation of the Solutions A standard solution of MCFG and a solution containing an internal standard substance (IS), FR195743, were supplied by Fujisawa Pharmaceutical Co., Ltd. MCFG was dissolved in ethanol at a concentration of 100 μg/ml, and the standard stock solution thus prepared was stored at −20°C. The IS was dissolved in 0.02 mol/l potassium dihydorgenphosphate/acetonitrile (50/50, v/v) at a concentration of 100 μg/ml, and the IS stock solution thus prepared was stored at 4°C. The standard stock solution was diluted with ethanol to make standard working solutions at a concentration of 25, 10, 2.5, 1, 0.25, and 0.05 μg/ml, respectively. The IS stock solution was diluted with 0.02 mol/l potassium dihydorgenphosphate/acetonitrile (50/50, v/v) to make an IS working solution at a concentration of 0.25 μg/ml.

Determination of Plasma MCFG by the Internal Standard Method Plasma samples (50 μl) from a healthy volunteer without MCFG treatment was transferred to a plastic microcentrifuge tube, and 50 μl of the standard working solution, 50 μl of the IS working solution, 50 μl of acetonitrile were added, and then mixed vigorously for 10 s. These solutions were centrifuged at 10000 rpm for 1 min, and
100 μl aliquot of the supernatant was mixed with 200 μl of 0.02 mol/l potassium dihydogenphosphate/acetonitrile (50/50, v/v). The mixture was filtered with a filter membrane (Millex-LG, 0.45 μm), and 75 μl aliquot of the filtrate was determined by high performance liquid chromatography (HPLC). The calibration curve was obtained by the peak height ratio of MCFG vs. the IS. Plasma samples (50μl) from the patients treated with MCFG was determined by the internal standard method.

**HPLC Analytical Conditions** HPLC was performed using a TSK gel ODS-80Ts column (4.6 mm×150 mm) as an analytical column and a TSK guard gel ODS-80Ts (3.2 mm×15 mm) as a guard column. The mobile phase of 0.02 mol/l potassium dihydogenphosphate/acetonitrile (56/44, v/v) was pumped at a flow rate of 1 ml/min, and column temperature was kept at 50 °C. Fluorometric detection was carried out with a RF-530 fluorometer (Shimadzu) at an excitation wavelength of 273 nm and a fluorescence wavelength of 464 nm.

**Statistical Analysis** We used the SPSS®11.5 software for Windows®.

**RESULTS**

The patient background, dose, blood level, total bilirubin level (T.Bil), serum creatinine clearance (CCr), C/D value (blood MCFG level/MCFG dose), and presence or absence of FK-506 are shown in Table 1 (including overlapped patients). CCr was calculated by the Cockcroft-Gault simple calculation method.

**Patient Background** The underlying diseases in the 29 subjects consisted of acute myeloblastic leukemia (AML) in 13 patients, acute lymphoblastic leukemia (ALL) in 3 patients, chronic myeloblastic leukemia (CML) in 1 patient, non-Hodgkin’s lymphoma (NHL) in 5 patients, multiple myeloma (MM) in 1 patient, plasma cell leukemia (PCL) in 1 patient, aplastic anemia (AA) in 1 patient, myelodysplastic syndrome (MDS) in 3 patients, and myelofibrosis (MF) in 1 patient.

**Dosage** In the 29 patients (40 samples), MCFG was dissolved in 100 ml of 0.9% sodium chloride injection, and intravenously infused over 1 h once a day. As shown in Table 1, we used the SPSS®11.5 software for Windows®.
the doses were 50 mg in 6 samples, 75 mg in 7 samples, 100 mg in 15 samples, 150 mg in 10 samples, and 300 mg in 2 samples.

**Blood Level** In the 40 samples, we analyzed the relationship between the dose of MCFG and the blood level of MCFG. As shown in Fig. 1, there was a correlation between the dose of MCFG and the blood level of MCFG ($r=0.729$, $p<0.001$). As shown in Fig. 2, we analyzed the relationships between T.Bil/CCr and the C/D value (the number of patients: refer to the figure). There was no correlation between CCr and the C/D value. However, there was a correlation between T.Bil and the C/D value ($r=0.458$, $p<0.01$).

In addition, as shown in Fig. 3, we compared the dose of MCFG, T.Bil, and CCr between patients in whom MCFG was combined with tacrolimus (FK-506), which was administered as an immunosuppressive agent after hematopoietic stem cells transplantation, and patients to whom only MCFG was administered. There were no significant differences between the combination therapy group and the non-combination therapy group (for the number of patients refer to the figure). As shown in Fig. 4, we compared the blood level of MCFG between the combination therapy group and the non-combination therapy group. There was no significant difference between the two groups (for the number of patients refer to the figure).

**DISCUSSION**

In our results, there were no marked changes in the blood level of MCFG even when it was administered to patients...
with mild kidney dysfunction; we did not consider it necessary to adjust the dose of this agent. Among conventional antifungal agents, the doses of some agents must be adjusted for patients with kidney dysfunction; therefore, MCFG may be relatively safely administered to patients with kidney dysfunction. When MCFG was administered to patients with biliary stasis, the blood level of MCFG increased, suggesting the necessity of regulating the dose. Previously, it has been reported that the main metabolic/excretory route of MCFG is the liver, and our results clinically support the hypothesis.

A post-marketing surveillance of MCFG has revealed that some patients developed liver/kidney dysfunction, which was not observed early after MCFG became commercially available. In 2 of the 17 patients who were treated with MCFG in our previous study and in 2 patients in whom the blood level of MCFG was measured in this study, this agent was discontinued due to liver dysfunction. The mechanism of liver dysfunction related to MCFG remains to be clarified. However, as there was no correlation between liver dysfunction and the blood level of the drug, various metabolites of MCFG may be involved in the development of the side effect (unpublished data). In the future, the blood level of MCFG should be measured in patients with side effects, including liver dysfunction, during administration of MCFG, and the correlation between metabolites and the incidence of side effects should be investigated.

There were no significant differences in gender or age between the combination therapy group and the non-combination therapy group (unpublished data), suggesting that FK-506 does not influence the blood level of MCFG. Previously, fluconazole (FLCZ) had been mainly prescribed for prophylactic administration of antifungal agents in the hematological field. However, the limitations of FLCZ included the presence of Aspergillus, Candida glabrata, and Candida krusei with low drug sensitivity, and frequent drug interactions. When FLCZ-resistant mycosis was suspected during prophylactic administration of FLCZ, the agent was switched to amphotericin B (AMPH-B). Long-term prophylactic administration of AMPH-B caused side effects such as kidney hypofunction, fever, and nausea, and there have been some serious cases. However, since the appearance of MCFG, MCFG has been increasingly selected as the first option for prophylactic administration of antifungal agents in the hematological field, and considering sensitivity and side effects, this agent has become essential for treatment.

In the future, the metabolism, optimal blood level, interactions of MCFG, and combination therapy with MCFG, which will be more routinely used, should be investigated in a larger number of patients. We are going to perform various clinical examinations.

CONCLUSION

Our clinical study suggests that MCFG can be relatively safely administered to patients with mild kidney hypofunction. In patients with biliary stasis-type liver hypofunction, the dose should be regulated. In combination therapy with FK-506, FK-506 did not influence the blood level of MCFG.

REFERENCES


![Fig. 4. Relationship between the Presence or Absence of FK506 and the Blood Level of MCFG](image-url)

There was no significant difference in the blood level of MCFG between the FK-506-treated group and the untreated group.