Monitoring of Blood Cyclosporine Concentration in Steroid-Resistant Nephrotic Syndrome

Masayo Naito, Takashi Takei, Aya Eguchi, Keiko Uchida, Ken Tsuchiya and Kosaku Nitta

Abstract

Objective  Cyclosporine has been used for patients with nephrotic syndrome. Because of substantial inter- and intra-patient variability and a narrow therapeutic window, drug monitoring of cyclosporine is mandatory. To confirm the therapeutic effects of a cyclosporine microemulsion (CSAME), the absorption profile of the agent after preprandial administration was determined in steroid-resistant patients with refractory nephrotic syndrome.

Methods  Fourteen patients were enrolled into the study (mean age, 31.2±12; 6 men, 8 women). The patients received 1.5 mg/kg of cyclosporine 30 minutes before breakfast for 6 months. Blood cyclosporine concentration was measured 5 times serially: before administration (C0) and at 1-hour intervals until 4 hours after administration of cyclosporine (C1-C4). In addition, area under the concentration-time curve from 0-4 hours (AUC0-4) was calculated.

Results  After 6 months, CSAME showed marked improvement in proteinuria levels (8.3±4.8 g/day vs 0.8±0.4 g/day, p<0.001). No changes in serum creatinine and urea nitrogen levels were observed. In 83% of the patients, the CSAME peak concentration appeared within 1 hour after administration (C1). A strong positive correlation was noted between AUC0-4 and C1 (R²=0.90312) and C2 (R²=0.78431). The mean steroid (prednisolone) dose was 40 mg/day when CSAME treatment was started, but a lowering of the dose to 17.5 mg/day (p<0.001) was achieved at 6 months after CSAME therapy.

Conclusion  Preprandial administration of CSAME is effective in steroid-resistant patients with refractory nephrotic syndrome. C1 or C2, but not C0, was a good clinical marker for CSAME exposure.

Key words: cyclosporine, nephrotic syndrome, preprandial administration, absorption profile, proteinuria

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Introduction

Cyclosporine, a calcineurin inhibitor immuno-suppressive agent, has beneficial effects in the treatment of steroid-resistant and frequently relapsing nephrotic syndrome (1-4). The potency and duration of calcineurin inhibition by cyclosporine depend on the blood cyclosporine concentration and the period of time that effective inhibition is maintained (5, 6).

The high inter-individual variability in blood concentrations and narrow therapeutic index require dose adjustment according to trough concentrations (C0), 2 hours post-dose concentrations (C2) or the estimation of the area under the concentration curve (AUC) to avoid underdosage with treatment failure and overdosage with toxic effects after renal transplantation (7-10). To prevent cyclosporine-induced nephropathy, however, maintaining the appropriate blood cyclosporine concentration within a narrow therapeutic window appears to be essential.

Recently, cyclosporine microemulsion (CSAME; Novartis Pharma, Basel, Switzerland) was introduced. When CSAME was administered postprandially, cyclosporine peak concentration appeared within 1-2 hours after administration, which is defined as high absorption. In some patients, however, cyclosporine absorption is delayed and the peak concentration does not occur within the first 2 hours after dosing. This delayed peak effect is defined as low cyclosporine absorption.
Table 1. Preprandial Absorption Profile of Cyclosporine Emulsion

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<th>C2</th>
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AUC0-4, area under the blood concentration-time curve up to 4 hours after cyclosporine administration

Preprandial administration of CSAME provided a more stable absorption profile in adult patients with refractory nephrotic syndrome (11). However, the dose adjustment of CSAME and the clinical efficacy of this treatment have not been completely investigated. The aim of our study was to examine the effects of preprandial administration of CSAME on the clinical parameters in patients with refractory steroid-resistant nephrotic syndrome.

**Subjects and Methods**

From January 2004 to March 2006, we enrolled 14 patients with refractory steroid-resistant nephrotic syndrome who were prescribed cyclosporine as the CSAME. Their mean age was 31.2±12.6 years in 6 men and 8 women. Their primary diseases were minimal-change nephrotic syndrome in 10 patients and focal segmental glomerulosclerosis in 4 patients. The research protocol was approved by the institutional review board of the hospital of Tokyo Women’s Medical University, and all patients were given written informed consent. Definitions were complete remission, reduced urinary protein excretion to <0.1 g/day for 1 month of prednisolone therapy (1 mg/kg/day); partial remission, decreased urinary protein excretion by >50% from the baseline on initial presentation; steroid-resistant nephrotic syndrome, no improvement in proteinuria after 1 month of prednisolone therapy (1 mg/kg/day); steroid-dependent nephrotic syndrome, at least two relapses during alternate-day steroid treatment or within 14 days after stopping steroid therapy.

All patients studied had steroid-resistant nephrotic syndrome before CSAME treatment. That is, no improvement in proteinuria after 1 month of steroid therapy (1 mg/kg/day). In minimal change nephrotic syndrome, the patients were steroid-resistant after at least two relapses. The mean dose of steroid was 40 mg/day at the start of CSAME treatment. In focal segmental glomerulosclerosis, all patients studied were steroid-resistant.

Cyclosporine was administered 30 minutes before breakfast in all patients. Blood monitoring of cyclosporine was performed once in 5 patients, twice in 6 patients and three
times in 3 patients as shown in Table 1. We measured blood cyclosporine concentrations 5 times serially: before administration (C0), and at 1-hour intervals for 4 hours after cyclosporine administration (C1, C2, C3, and C4). Cyclosporine concentration was measured by the fluorescence polarization immunoassay method as previously described (12), with use of the CyA-dyna-pack and TDx analyzer (Abbott Laboratories, Abbott Park, IL). Based on these measurements, AUC\textsubscript{0-4} was calculated by the trapezoidal method (13). Based on the cyclosporine dosing recommendations for patients after renal transplantation (14), we aimed for a cyclosporine concentration of 50-150 ng/mL for C0 and 600-800 ng/mL for C2, and the AUC\textsubscript{0-4} at 1,600-2,000 ng·hour/mL. Clinical parameters such as blood pressure, daily proteinuria, and serum levels of total protein, albumin, urea nitrogen, creatinine and total cholesterol before and 6 months after the start of this clinical study. Clinical efficacy of cyclosporine was determined from changes in proteinuria, total serum protein, serum albumin, total cholesterol and steroid dosage after 6 months of cyclosporine treatment compared with pretreatment. Serum creatinine and urea nitrogen levels served as the indices of cyclosporine nephrotoxicity.

All values are expressed as means ± SD. The Pearson correlation coefficient was used to analyze the correlation between AUC\textsubscript{0-4} and C0, C1, and C2 in those who exhibited a low-absorption pattern and in those with a high-absorption pattern. Student unpaired t test was used to evaluate differences in cyclosporine dosage, C0, C1, C2, and AUC\textsubscript{0-4}. A p value less than 0.05 was considered to indicate a significant difference.

**Results**

A preliminary study was performed within 1 week from the start of cyclosporine treatment. CSAME was administered (75 mg) once daily before and after breakfast in a patient with steroid-resistant nephrotic syndrome. As shown in Fig. 1, peak level was higher in preprandial than postprandial administration of CSAME in this patient. Then we started the current study.

Changes in the clinical parameters at 6 months of treatment compared with pretreatment values for each patient are shown in Table 2. Treatment with CSAME improved total serum protein (4.4±0.9 mg/dL vs 6.3±0.6 mg/dL, p<0.001), albumin (2.3±0.8 g/dL vs 4.1±0.5 g/dL, p<0.001) and total cholesterol (432.9±144.1 mg/dL vs 235.8±76.3 mg/dL, p<0.01). In addition, all the patients treated with cyclosporine showed marked improvement in proteinuria levels (8.3±4.8 g/day vs 0.8±0.4 g/day, p<0.001). These values improved regardless of the histological diagnosis or duration of nephrotic syndrome. Complete remission was achieved 8 of the 14 patients and the remaining patients were judged to achieve partial remission.

The initial dosage of CSAME and the C0, C1, C2, C3, C4 and AUC\textsubscript{0-4} are shown in Table 1. The mean dosage of CSAME at the start of treatment was 1.57 mg/kg/day (50-200 mg/day). The mean AUC\textsubscript{0-4} was 1416.5±445.09 ng·h/
mL. The mean C0 level was 41.17±19.9 ng/mL. In 83% of the patients, the cyclosporine peak concentration appeared within 1 hour after administration (C1). Figure 2 shows the correlation between AUC0-4 and C0, C1 and C2. A strong positive correlation was noted between AUC0-4 and C1 \(R^2=0.90312\) and C2 \(R^2=0.78431\). The mean steroid (prednisolone) dose was 40 mg/day when CSAME treatment was started, but a lowering of the dose to 17.5 mg/day (p<0.001) was achieved at 6 months after CSAME therapy. Figure 3 shows the absorption profile measured within 1 week from the start of CSAME treatment. The mean peak cyclosporine levels (Cmax) was 682.09±227.81 ng/mL. Peak levels were reached at C1 19 times in 13 patients, C2 3 times in 2 patients, C3 once in one patients and C4 3 times in 2 patients.

There were no changes in serum creatinine or urea nitrogen levels before and after 6 months after CSAME treatment (0.8±0.2 mg/dL vs 0.8±0.3 mg/dL, p=0.36 and 18.2±0.8 mg/dL vs 18.4±0.9 mg/dL, p=0.42, respectively) (Table 2). In addition, no patient showed serious worsening of renal function during the treatment. No change in systolic or diastolic blood pressure was observed before and after CSAME treatment. Moreover, there were no clinically significant adverse effects due to CSAME during the observation period.

**Discussion**

The present study was designed to investigate whether preprandial administration of CSAME would result in disease remission in steroid-resistant patients with refractory nephrotic syndrome. First, we identified that complete remission was achieved 30% of the patients and the remaining patients were judged to achieve partial remission. Second, the single sampling point that best correlated with AUC0-4 was C1 or C2, and there was no correlation between AUC0-4 and C0. Third, mean C0 levels were low in all patients, indicating the inability of C0 to predict cyclosporine absorption status. Finally, CSAME administration reduced steroid dosages prescribed in these patients.

There have been a few studies that suggest optimal administration method of CSAME in the treatment of nephrotic syndrome based on pharmacokinetic parameters. The pharmacokinetic profile provides an indicator of the appropriate cyclosporine administration to obtain a sufficient ef-
fect and to avoid adverse effects. Cyclosporine concentration traditionally is measured just before drug administration, which is termed the trough level (C0). This approach is used because reproducibility is good for the outpatient setting. Recently, the AUC_{0-4} was found to correlate better with clinical effects in kidney and liver transplant recipients (15, 16). AUC_{0-4} correlated well with C2 but not with C0; therefore, C2 monitoring has been proposed to be a more sensitive tool for monitoring cyclosporine absorption (17, 18).

Kusaba et al (11) reported the absorption profile of CSAME in adult patients with nephrotic syndrome. Preprandial administration provided a more stable absorption profile of cyclosporine compared with postprandial administration. From the correlation with AUC_{0-4}, they concluded that C2, and not C0, is a reliable marker for monitoring cyclosporine exposure. When CSAME is administered preprandially, the bile acids and the drug would be effectively mixed before food enters the upper gastrointestinal tract, which may result in improved cyclosporine absorption (19).

Takeda et al (20) have recently reported the benefits of preprandial once-daily administration of CSAME in nephrotic syndrome. Preprandial administration provided a more stable absorption profile of cyclosporine compared with postprandial administration. Some patients with nephrotic syndrome had slow absorption even when given cyclosporine in the microemulsion formulation. When the dose of cyclosporine was adjusted according to the absorption profile, a strong correlation was noted between AUC_{0-4} and C2. In the current study, the correlation coefficient was higher between AUC_{0-4} and C1 than between AUC_{0-4} and C2. This may be due to the lack of adjustment of cyclosporine dosage according to absorption profile. Dose adjustments based on the absorption profile are likely a useful tool due to poor oral absorption of the drug and low oral bioavailability in patients with nephrotic syndrome.

The present study has several limitations. First, we did not have a control group as postprandial administration of CSAME. Only one patient was examined for the absorption profile of CSAME after administration once daily before and after breakfast. Based on the results of a previous report (11, 20), we would support their data, suggesting that preprandial administration of CSAME is useful to achieve an absorption profile in steroid-resistant nephrotic syndrome.

Second, we measured the absorption profile of CSAME in the same patients several times. This clinical approach seems to be not adequate for the determination of absorption profile of CSAME. However, the absorption profile is not stable even if the same dose of CSAME is administered to the same patient in a different study period. Thus, we propose that the absorption profile should be measured at least twice during the study period.

In conclusion, the current study showed the efficacy of preprandial administration of CSAME in steroid-resistant patients with refractory nephrotic syndrome. C1 or C2, but not C0, was a good clinical marker for cyclosporine exposure (measured as AUC_{0-4}). Long-term observation is necessary to clarify the clinical advantage of preprandial administration such as the reduction of urinary protein or the prevention of relapse of refractory nephrotic syndrome.

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References
2. Iida HNT, Sakai N, Aoki S. Effect of cyclosporin therapy on idiopathic membranous nephropathy presented with refractory ne-

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http://www.naika.or.jp/imindex.html