Tacrolimus Metabolite M-III May Have Nephrotoxic and Myelotoxic Effects and Increase the Incidence of Infections in Kidney Transplant Recipients

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ABSTRACT

Background. Tacrolimus (Tac) is one of the most commonly used immunosuppressive drugs after solid organ transplantation. Eight Tac metabolites have been described, but their clinical importance remains unclear. The aim of this study was quantification of the 2 major Tac metabolites, 13-O-demethyl (M-I) and 15-O-demethyl (M-III), in kidney transplant recipients and to link them with parameters of kidney and liver function, peripheral blood cell counts, and infection incidence.

Methods. In 81 kidney transplant recipients, concentrations of Tac, M-I, and M-III were measured with the use of liquid chromatography combined with tandem mass spectrometry (LC-MS-MS).

Results. There was a negative correlation between M-III levels and estimated glomerular filtration rate (eGFR; $r = -0.244$; $P < .05$). Also, a negative correlation between M-III concentrations and red blood cell count (RBC) was found ($r = -0.349$; $P < .05$). Neither concentrations of Tac nor of M-I correlated with eGFR or RBC. M-I, M-III, and Tac were not related to alanine aminotransferase activity. Significantly higher Tac and M-III concentrations in the group with all types of infections in comparison with the group without infections were observed (6.95 ± 2.09 ng/mL vs 5.73 ± 2.43 ng/mL [$P = .03$] and 0.27 ± 0.17 ng/mL vs 0.20 ± 0.11 ng/mL [$P = .04$], respectively).

Conclusions. The results suggest that higher concentrations of M-III may have a nephrotoxic or myelotoxic effect and result in higher incidence of infections. Further studies are needed to confirm if monitoring of M-III could minimalize adverse effects such as nephrotoxicity or infections.

TACROLIMUS (TAC) is one of the most commonly used immunosuppressive drugs after solid organ transplantation. Owing to its narrow therapeutic window, Tac requires therapeutic drug monitoring. In clinical practice, monitoring of predose trough blood concentrations is routinely used [1]. Unfortunately, it is not always sufficient for guiding optimal long-term dosing of this drug. Adverse effects of Tac include nephrotoxicity, neurotoxicity, diabetogenic effect, and infection. Tac undergoes extensive

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metabolism in the liver. It is metabolized by hepatic and intestinal cytochrome P450 enzymes, especially cytochrome P450 3A (CYP3A), to at least 8 metabolites [2]. Four of them are major metabolites of varying immunosuppressive activity: 13-O-demethyl (M-I), 31-O-demethyl (M-II), 15-O-demethyl (M-III), and 12-hydroxyl (M-IV) [3]. M-I to M-IV are first-generation metabolites, formed directly from the parent drug. M-V to M-VIII are formed from the 1st-generation metabolites and are considered to be 2nd-generation metabolites. Dubbelboer et al found the mean concentrations of M-I, M-II, and M-III to be 10%, 4%, and 6%, respectively, of the Tac concentration [4]. Active metabolites could be responsible for drug toxicity. The toxic properties of Tac metabolites are unknown and their clinical importance remains unclear.

The aim of the present study was the assessment of concentrations of the 2 major Tac metabolites, M-I and M-III, with the use of liquid chromatography combined with tandem mass spectrometry (LC-MS-MS) in kidney transplant recipients. We tried to correlate metabolite levels with parameters of kidney and liver function, peripheral blood cell counts, and infection incidence.

METHODS

The study protocol was approved by the local Ethics Committee and was in accordance with the revised Declaration of Helsinki. Outpatients who gave their written informed consents to participate in the study were included. The study included 81 stable kidney transplant recipients from whom 2 mL of blood was collected during routine visits. Blood was taken just before the administration of the morning dose of Tac (trough concentration) and after fasting for $\geq$8 hours. Blood was collected in EDTA tubes and stored at −80°C until the time of determination of Tac and its metabolite concentrations by means of LC-MS-MS.

Blood sampling was accompanied by the collection of relevant laboratory and clinical data. Renal function was assessed with the use of estimated glomerular filtration rate (eGFR) with the use of the Modification of Diet in Renal Disease formula [5]. Liver function was assessed by means of alanine transaminase (ALT) activity. White blood cell count (WBC), red blood cell count (RBC), and blood platelet count (PLT) were also assessed. Anemia was diagnosed if the blood hemoglobin was <120 g/L (women) or <130 g/L (men). The diagnosis of infection was made on the basis of patients' signs and symptoms and results of laboratory and microbiologic tests.

Chemicals

Tacrolimus and ascomycin (internal standard) were acquired from Toronto Research Chemicals (North York, Ontario, Canada), and M-I was a gift from Astellas Pharma (Osaka, Japan). All stock solutions were prepared in methanol and stored in −20°C. LC-MS–grade methanol, high-performance LC (HPLC)–grade methanol, HPLC-grade acetonitrile, and formic acid were purchased from JT Baker. Ultrapure water was produced with the use of a water purification system (Mili-Q, Millipore, Milford, Massachusetts). Zinc sulfate monohydrate was purchased from Sigma-Aldrich (St Louis, Missouri), and analytic-grade ammonium acetate was obtained from POCH (Gliwice, Poland).

Sample Preparation

Whole blood was collected in EDTA tubes and stored at −80°C until the time of determination of Tac and its metabolites concentrations by means of an LC-MS-MS method. Samples were prepared according to a previously described protocol [6]. A 30-μL aliquot was injected into the LC-MS-MS system.

Instrumentation

The analyses were performed with the use of Waters Acquity Ultra Performance Liquid Chromatograph coupled with Waters TQ-S triple-quadrupole mass spectrometer. Waters MassLynx software was used for the instrument control and data acquisition, and Waters TargetLynx was used to processed data.

Analyses

Separation was performed with the use of a Waters BEH C18 column (1.7 μm; 2.1 mm × 50 mm). The column was thermostatted at 50°C. Mobile phase A consisted of 2 mmol/L ammonium acetate with 0.1% formic acid (v/v) in water, and mobile phase B consisted of 2 mmol/L ammonium acetate with 0.1% formic acid (v/v) in methanol. The gradient scheme was 40% B initially, increased to 70% B at 0.8 minutes, and then to 98% B at 1.4 minutes with suspension for 0.4 minutes. At 3.3 minutes, the mobile phase B reverted to 40%. The total analysis time with reequilibration was 3.5 minutes. LC-MS-MS analysis was performed in a positive electrospray ionization mode, and the mass spectrometer was operated in a multiple-reaction monitoring mode. For all analyses, MS optimized settings were as follows: capillary voltage = 1.5 kV; desolvation temperature = 200°C; cone gas flow = 150 L/h; desolvation gas flow = 800 L/h; source temperature = 150°C. The calibration curve ranges were 1–40 ng/mL for Tac and 0.01–1.5 ng/mL M-I. Concentration of M-III was quantified with the use of the M-I calibration curve. Mean R^2 coefficients of a calibration curve from 7 calibration samples were $\geq$0.97. The method showed good intra-assay and interassay precision: <10%.

The Kolmogorov-Smirnov test was used to assess variables' normality. Continuous data were described as mean ± SD for normal distribution and as median and range for data with nonnormal distribution. The Student t test and Mann-Whitney U test were applied to determine the differences between normally and nonnormally distributed variables, respectively. Correlations between the parameters were calculated with the use of the Pearson and Spearman correlation coefficient for normally and nonnormally distributed variables. A P value of <.05 was considered to be significant. The statistical analyses were performed with the use of Statistica 12 (Statsoft, Tulsa, Oklahoma).

RESULTS

Patients' demographics and concentrations of Tac, M-I, and M-III are presented in Table 1. There was a negative correlation between M-III levels and eGFR (Table 2). Also, we found a negative correlation between M-III concentrations and RBC (Table 2). Neither Tac nor M-I correlated with eGFR and RBC. M-I, M-III, and Tac were not related to ALT activity. We observed significantly higher Tac and M-III concentrations in the group with all types of infections compared with the group without infections (Table 3). In the group with anemia, there were higher Tac and M-III concentrations than in patients without anemia (Table 3).