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Plasma and intracellular pharmacokinetic–pharmacodynamic analysis of mycophenolic acid in de novo kidney transplant patients

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ABSTRACT

Objectives: Little is known about the correlation between the inosine-monophosphate dehydrogenase (IMPDH) activity and mycophenolic acid (MPA) concentrations in peripheral-blood-mononuclear cells (PBMCs), where the drug is acting. The aim of this study was to analyze the relationship between plasma or PBMC MPA levels, as pharmacokinetic (PK) markers, and the intracellular IMPDH enzyme activity, as a pharmacodynamic (PD) biomarker, in kidney transplantation.

Design and methods: Forty de novo renal transplant patients were enrolled in this prospective study. The sampling was performed on the day before transplantation and at T_0, $T_{1.5}$ and $T_{3.5}$ following the morning dose, on days 2, 4 and 10 post-transplantation. All subjects were treated with a fixed MMF dose (500 mg twice-a-day). IMPDH activities were determined by HPLC, and MPA plasma or PBMC concentrations were obtained by LC–MSMS.

Results: Important inter-patient variability was observed both for the PK and PD biomakers. Pre-dose IMPDH activity, surprisingly, increased during the 10 days post-transplantation. As expected, a significant inverse relationship was found between IMPDH activities and MPA concentrations in both plasma and PBMCs. A significant correlation was found between plasma and PBMC MPA values. Maximum IMPDH inhibition was found mostly at $T_{1.5}$, before returning to its pre-dose levels at $T_{3.5}$. IMPDH inhibition at $T_{1.5}$ better correlated with plasma MPA $AUC_{0-3.5}$ ($p = 0.027$) than with PBMC $AUC_{0-3.5}$ ($p = 0.323$). Mean MPA plasma concentrations paralleled the enzyme inhibition profiles and decreased strongly at $T_{3.5}$, whereas the decreasing slope of MPA concentrations in PBMCs appeared slower.

Conclusions: These findings suggest that PBMC MPA concentrations do not provide any better correlation with the IMPDH activity than plasma MPA values, most likely due to the correlation between plasma and PBMC MPA levels and to the important interpatient variability both in MPA levels and enzyme activities.

Introduction

Mycophenolic acid (MPA), the active drug moiety of two pro-drugs (mycophenolate mofetil (MMF) and EC-mycophenolate sodium), is a potent, selective, and non-competitive reversible inhibitor of the inosine 5′-monophophate dehydrogenase (IMPDH), a rate-limiting enzyme in the de novo synthesis of guanosine nucleotides selectively required for lymphocyte proliferation [1,2]. The cytostatic effect of the drug is more potent on lymphocytes (expressing the type II isoform of IMPDH) than on any other cells. MPA is rapidly released from MMF by esterases in the intestine and blood, before penetrating in the cells [2]. MPA is predominantly metabolized by uridine diphosphate glucuronosyl transferase 1A isoform (UGT1A) to the inactive form 7-O-MPA-glucuronide (MPAG) [3,4], and by UGT2B7 to the pharmacologically active form acyl-glucuronide of MPA (AcMPAG) in various tissues including the liver and the gut [4]. MPAG is partly excreted into the bile by the multidrug resistance-associated protein 2 (MRP2) [5,6]. In the gastro-intestinal tract, MPA is again released contributing to an enterohepatic recirculation [7] and an increase in bioavailability. Because of the difficult equilibrium between under- and over-immunosuppression, therapeutic drug monitoring (TDM) of MPA has been proposed to optimize drug dosage avoiding potential hematologic and digestive side effects. MPA monitoring is generally based on the determination of the plasma MPA trough concentration (C_0), or on AUC_{0-12}. This last pharmacokinetic (PK) marker displayed better pharmacodynamic (PD) predictive performances than C_0 [8,9]. The measurement of the IMPDH activity in peripheral blood mononuclear cells (PBMCs) or in whole blood [10–12] has been proposed as a PD biomarker

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of MPA efficacy. A relationship between MPA plasma levels and IMPDH activity has been shown. Up to now, little is known about the potential interest to determine MPA concentrations within the PBMCs, mostly lymphocytes where the drug is acting, and about the relationships between plasma or PBMC drug concentrations and IMPDH activity. It has been demonstrated that more than 98% of the drug remains in the plasma compartment, mainly bound to albumin, and that a minor fraction reaches the mononuclear cell compartment [2,13–16]. It could therefore be hypothesized that the IMPDH inhibition, and likely the clinical efficacy, could be primarily related to PBMC drug levels. The aim of this investigation is to analyze the MPA PK–PD relationship both in plasma and PBMCs, with the IMPDH inhibition, in an attempt to improve the patient follow-up.

Materials and methods

Patient population

Forty de novo kidney transplant recipients, ranging from 21 to 67 years, were enrolled and followed during the 10 first days following transplantation. They were treated with a fixed MMF dose (500 mg twice-a-day) in combination with tacrolimus (Advagraf®) and a fixed methylprednisolone dose (8 mg Medrol® twice-a-day). Adjustment of the Advagraf® dose was based on whole blood drug concentrations. The study was approved by the local Ethics Committee and all patients were given their informed consent.

Pharmacokinetic–pharmacodynamic (PK–PD) study

PK assessments were obtained by determining MPA plasma and PBMC concentrations, whereas PD evaluation was based on the measurement of IMPDH activity in PBMCs. PK–PD profiles were obtained during the early post-transplant period (days 0–10). For the PK sampling, samples were collected at time 0 (T_0), 1.5 h ($T_{1.5}$) and 3.5 h ($T_{3.5}$) following the morning MMF dose on days 2, 4 and 10 after transplantation. For the PD sampling, IMPDH activity was determined before transplantation (baseline), and again at T_0, $T_{1.5}$ and $T_{3.5}$, on days 2, 4 and 10. A deviation of ± 5 min was accepted for all sampling. Heparin and EDTA blood samples were obtained for the determinations of IMPDH activity and MPA concentration in PBMCs and plasma, respectively. All samples were processed at room temperature (RT) within 12 h after collection. Reduced Area under the time–concentration Curve ($AUC_{0-3.5}$) and Area under the pharmacodynamic-Effect time Curve ($AEC_{0-3.5}$) were calculated by the linear trapezoidal rule. Demographic and biological data are displayed in Table 1.

Table 1
Demographic and biological characteristics of the kidney transplant patients (N = 40) at day 10 post-transplantation.

Patient characteristics	Mean (range)
Age (year)	50.89 (21–67)
Weight (kg)	68.27 (39–108)
Height (cm)	168.38 (150–190)
Gender, M/F	21/19
Albumin (g/L)	43.6 (33.0–52.0)
Serum creatinine (μmol/L)	212.1 (51.2–1211.1)
Total bilirubin (μmol/L)	9.23 (5.13–18.8)
Plasma protein (g/L)	69.7 (50–89.7)
Hematocrit (%)	30.3 (23–42)
MPA plasma trough level (mg/L)	1.34 (0.21–2.47)
MPA PBMC trough level (ng/10^7 cells)	2.59 ± 2.68 (0.3–12.75)
Tacrolimus whole-blood trough level (μg/L)	12.3 (5.8–25.3)
IMPDH pre-dose activity (nmol/h/mg protein)	8.93 (5.27–18.95)

Analytical methods

Reagents

Acetonitrile and methanol were of ultra-performance liquid chromatography/mass spectrometry grade from J.T. Baker (Deventer, Netherlands). Triethylammonium phosphate buffer 1 M (pH 3), Dulbecco's Phosphate Buffered Saline (PBS), IMP, NAD^+, XMP and bovine serum albumin were obtained from Sigma Life Science and Sigma-Aldrich Chemicals (St. Louis, Switzerland; Steinheim, Germany). MPA and MPA-d3 were the gifts from Toronto Research Chemicals Inc. (North York, Canada). Leucosep® tubes with Ficoll-Paque solution were obtained from Greiner Bio-One (Wemmel, Belgium). Potassium chloride, potassium carbonate, sodium dihydrogen phosphate, acetate ammonium and formic acid were obtained from Merck (Darmstadt, Germany).

Measurement of IMPDH activity in PBMCs

PBMCs were isolated from whole blood using Leucosep® tubes according to the manufacturer's instructions, and the procedure of Glander et al. [17] after minor changes. Lithium heparin blood samples (2.5 mL) were carefully poured into a Leucosep® tube 12 mL and centrifuged for 20 min at 1200 *g* at RT without brakes. After discarding the plasma layer fraction, PBMCs were harvested and washed with 10 mL of PBS and subsequently centrifuged at 1000 *g* for 20 min at 4 °C. The cell pellet was resuspended in 250 μL of water (HPLC grade, 4 °C) and stored at −80 °C. After thawing the frozen cell pellet, insoluble fragments were removed by centrifugation at 1200 *g* for 5 min. The supernatant lysate was used for enzymatic assay (100 μL) and protein (20 μL) determination. The protein concentrations were determined by the Lowry method.

The IMPDH activity was measured by HPLC. 300 μL of reaction environment, containing 40 mM sodium dihydrogen phosphate pH 7.4, 100 mM potassium chloride, 0.5 mM IMP, and 0.5 mM NAD^+, were incubated at 37 °C for 5 min. The kinetic enzymatic reaction occurred within 3 h after adding 100 μL supernatant lysate to the 300 μL of reaction environment. The enzymatic reaction was stopped by transferring sequentially 50 μL of reaction mixture into 1.5 mL Eppendorf tubes containing 12.5 μL of 10% perchloric acid maintained on ice, at 0, 45, 90, 120, 150 and 180 min. Centrifugation of the Eppendorfs was performed at 14,000 *g* at 4 °C for 5 min in order to separate proteins. Subsequently, 45 μL of supernatant was neutralized with 4 μL of 1.5 M potassium carbonate and then centrifuged again at 14,000 *g* at 4 °C for 5 min. The supernatants were stored at −80 °C until assay or immediately injected into the HPLC column to determine the produced XMP.

Normalization of the enzyme activity is usually obtained by cell counting or protein concentration, with generally a preference for the last approach [8]. A third method has been proposed by Glander et al., based on the intracellular AMP concentration [17]. We have tested these two last normalization methods (protein and AMP) in healthy volunteers (n: 39) and patient groups (n: 25), with similar performances. The protein normalization method has been selected for this study because of its widespread use and slightly better reproducibility.

Chromatographic analysis for XMP was carried out using a Lichrocart C18 column (125 × 4 mm, 5 μm) maintained at 40 °C on a HPLC Alliance 2695 instrument (Waters) equipped with a photodiode array detector set at 254 nm. The mobile phase consisted of a mixture (2:98, v:v) methanol and triethylammonium phosphate buffer (pH 6.5) set at a flow rate of 1.0 mL/min. The isocratic separation was performed for 6 min after injection of a 10 μL sample, and was followed by an 8 min washing phase (50% MeOH). IMPDH activity in PBMCs was expressed as the ratio of produced XMP (μmol) versus protein concentration per incubation time (nmol XMP/h/mg of protein). The concentrations of XMP were determined using a linear calibration curve in the range of 0.5–80 μmol/L with a coefficient of determination (r^2) ranging from 0.997 to 0.999. Mean intra- and inter-assay imprecision levels were below 7%. The accuracy ranged from 94.22 to 114.19%. The recovery