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Pharmacokinetics and pharmacodynamics of mycophenolic acid in Nagase analbuminemic rats: Evaluation of protein binding effects using the modeling and simulation approach

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ABSTRACT

This study aimed to examine the pharmacokinetics and pharmacodynamics of mycophenolic acid (MPA) in Nagase analbuminemic rats (NARs) to evaluate the effect of protein binding on the associated inosine-5′-monophosphate dehydrogenase (IMPDH) activity. Free fractions of MPA in the control rats and NARs were 2.09 and 24.8%, respectively. Pharmacokinetic and pharmacodynamic parameters simultaneously obtained by the nonlinear mixed effects modeling program NONMEM explained reasonably well the concentrations of MPA and MPA glucuronide as well as IMPDH activity in both rats. NARs showed a higher clearance and a smaller volume of distribution based on the free MPA concentration than the controls did, besides the increase in free fraction. The half-maximal inhibitory concentration based on free MPA was estimated as 163 ng/mL for both rats. Simulations based on the obtained pharmacokinetic and pharmacodynamic parameters showed that the area under the IMPDH activity-time curve decreased non-linearly according to the increase in free fraction of MPA. In conclusion, the experimental data obtained from NARs followed by the modeling and simulation approach quantitatively clarified that the free MPA concentration was suitable for the biomarker of immunosuppressive effect of MPA. Dose adjustments based on the total MPA may cause unnecessary overexposure to MPA in patients with hypoalbuminemia.

1. Introduction

Mycophenolic acid (MPA) selectively inhibits inosine-5′-monophosphate dehydrogenase (IMPDH), which catalyzes the rate-limiting step in the *de novo* synthesis of guanine nucleotide and suppresses the proliferation of B and T lymphocytes [1]. MPA is clinically used to suppress acute rejection after solid organ transplantation as well as graft-*versus*-host disease in hematopoietic stem cell transplantation [2,3], in the combination with other immunosuppressants such as a calcineurin inhibitor, tacrolimus or cyclosporine.

MPA is metabolized by uridine 5′-diphospho-glucuronosyltransferase (UGT) isoforms in the liver to an inactive MPA glucuronide (MPAG), which is mainly eliminated through the urine [3]. The pharmacokinetics of MPA is affected by changes in hepatic or renal functions [4] and shows a large inter- and intraindividual variability [5]. Therefore, patients who have had transplants followed by MPA administration should be managed by therapeutic drug monitoring of plasma MPA concentrations. The consensus target exposure range of MPA has been proposed as an area under the concentration-time curve (AUC) from 0 to 12 h of 30–60 μg h/mL based on the total plasma concentration in patients with renal transplants [6]. The target exposure range of MPA is possibly influenced by changes in plasma albumin concentrations, since the free fraction of MPA was 1–2% [7].

IMPDH activity in peripheral blood mononuclear cells (PBMCs) has been suggested as a surrogate biomarker for the

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immunosuppressive effects of MPA, and pre-transplant IMPDH activity is associated with rejection in patients with renal transplants [8]. In a recent study, the area under the effect-time curve (AUEC) of IMPDH activity on day 21 after hematopoietic stem cell transplantation was found to be associated with cytomegalovirus reactivation, non-relapse mortality, and overall mortality [9]. Therefore, the measurement of IMPDH activity in addition to monitoring the AUC of total plasma MPA is considered an effective predictor of the clinical outcome of MPA therapy. However, little is known about the quantitative relationship between the free fraction of MPA in the plasma and IMPDH activity.

Nagase analbuminemic rats (NARs) are an established animal model for human familial analbuminemia [10]. NARs were used to examine the effect of decreased protein binding on the pharmacokinetics and pharmacodynamics of drugs having a high protein binding property, such as bumetanide [11], azosemide [12], and methotrexate [13]. NARs have also been utilized in the investigation of the toxicokinetics and toxicodynamics of clofibrate [14]. In this study, we constructed the simultaneous pharmacokinetic and pharmacodynamic model of MPA, and analyzed the experimental data obtained from NARs as well as control rats by the nonlinear mixed effects modeling method. The simulation study based on the obtained pharmacokinetic and pharmacodynamic parameters quantitatively evaluated the effect of protein binding on the IMPDH activity, as a biomarker of the pharmacodynamics of MPA.

2. Materials and methods

2.1. Chemicals

MPA and MPAG were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and Analytical Services International Ltd. (London, UK), respectively. Xanthosine-5′-monophosphate (XMP) disodium salt was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Adenosine-5′-monophosphate (AMP) sodium salt, inosine-5′-monophosphate disodium salt from yeast and nicotinamide adenine dinucleotide (NAD^+) were from Nacalai Tesque Inc. (Kyoto, Japan). All the chemicals used were of the highest grade available.

2.2. In vivo pharmacokinetic and pharmacodynamic study

Male Sprague-Dawley rats (control) and NARs (8- to 10-week-old and 9- to 11-week-old, respectively) were obtained from Japan SLC, Inc. (Osaka, Japan). Both groups of rats were anesthetized with intraperitoneal injections of 40 mg/kg pentobarbital sodium. A polyethylene tube was inserted into the femoral artery and vein. Then, MPA dissolved in 10% cremophor and 10% ethanol in saline was intravenously infused for 1 h via the femoral vein at doses of 0.5 or 5 and 5 or 15 mg/kg in the control and NAR groups, respectively. Blood samples (0.2 mL) were collected sequentially before and at 0.5, 1, 1.25, 1.5, 2, 2.5, 3, and 4 h after the start of MPA administration and were stored at 4 °C until completion of blood collection. The blood samples were centrifuged at 14,000 *g* for 5 min to obtain the plasma, and then plasma samples (100 μL) were acidified by adding 2 μL of 10% acetic acid for the assay of MPA and MPAG. The PBMCs, which were obtained from the residual blood samples by centrifuging at 1000 *g* for 15 min using Ficoll-Paque Premium 1.084 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), were frozen at −20 °C until measurements of IMPDH activity were performed. The free fraction of MPA and MPAG in both groups of rats was determined using plasma from blood samples collected 15 min after MPA administration. The plasma samples were ultrafiltrated (Amicon Ultra 30 K centrifugal filter devices, Merck Millipore Ltd., Carrigtwohill, Ireland) at 14,000 *g* for 10 min. The free MPA and MPAG concentrations were calculated by multiplying their respective total concentrations by the average of the free fraction of each drug for each group. All procedures were conducted in compliance with the Guidelines for Animal Experiments of the Kyoto University.

2.3. Analysis of cytochrome P450 enzyme (CYP) and UGT mRNA expressions using real-time polymerase chain reaction (PCR)

The total RNA was extracted from the liver using the RNeasy mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instruction and further quantitated using the BioSpec-nano spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 260 nm. The total RNA (1 μg) was reverse-transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems). The rat cDNAs were mixed with the forward and reverse primers of the target CYPs (CYP1A2, 2B2, 2C11, and 3A2), UGTs (UGT1A1, 1A2, 1A6, 1A7, and 1A8) or GAPDH and SYBR Green PCR master mix (Applied Biosystems). After an initial denaturation at 95 °C for 10 min, the amplification was performed by denaturation at 95 °C for 15 s and extension at 60 °C for 60 s, repeated for 50 cycles. The expression level of each mRNA was quantified by measuring the fluorescence intensity using the StepOnePlus real-time PCR system (Applied Biosystems) and was expressed as a ratio of GAPDH. The primer designs were referenced from various published articles [15–21], while the primer sequences and PCR product sizes were confirmed using primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

2.4. MPAG formation in rat liver microsomes

Liver microsomes from control rats and NARs were prepared according to a commonly used procedure [22]. MPAG formation by liver microsomes was studied by the previously reported method [23] with a slight modification. Liver microsomes (1 mg) was incubated in buffer containing 75 mM Tris-hydrochloric acid (pH 7.45) and 10 mM magnesium chloride. The total volume was 50 μL, and the final concentration of MPA was 5–640 μg/mL. After pretreated with alamethicin (50 μg/mg) for 15 min on ice, reactions were initiated by the addition of uridine 5'-diphospho-glucuronic acid (3 mM final concentration) and were allowed to proceed at 37 °C for 30 min. Reactions were stopped by the addition of two volumes of the internal standard methanol solution. The amount of MPAG formed was measured, and kinetic parameters, the Michaelis–Menten constant (K_m; μg/mL) and the maximum velocity (V_{max}; ng/mg protein per 30 min), were determined using a non-linear least squares method (WinNonlin 6.4; Pharsight, Mountain View, CA).

2.5. Analytical methods

Concentrations of MPA and MPAG were analyzed using a reverse phase liquid chromatography-tandem mass spectrometry according to the previously reported method [24]. The limits of determination were 2 and 20 ng/mL for MPA and MPAG, respectively.

The PBMC samples were used to measure IMPDH activity according to the previously reported method [24] with a slight modification. Briefly, thawed PBMCs diluted with 200 μL of water were vortexed and centrifuged at 1000 *g* for 2 min. The reaction was initiated by adding 50 μL of supernatant to 52 μL of incubation mixture consisting of 1.6 mM phosphate buffer (pH 7.4), 3.9 mM potassium chloride, 4.8 mM inosine-5′-monophosphate disodium salt, and 0.6 mM NAD^+ as final concentrations. The mixture was incubated at 37 °C for 120 min. The reaction was stopped by the addition of 5 μL of 2.5 M perchloric acid followed by 10 μL of 3 M