Acta Pharmaceutica Sinica B 2017;7(3):347–352

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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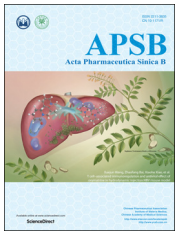

ORIGINAL ARTICLE

Nonlinear relationship between enteric-coated mycophenolate sodium dose and mycophenolic acid exposure in Han kidney transplantation recipients

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Received 19 July 2016; revised 21 September 2016; accepted 24 October 2016

KEY WORDS

Enteric-coated;
Mycophenolate sodium;
Mycophenolic acid;
Nonlinear dynamics;
Kidney transplantation;
Pharmacokinetics;
UPLC-UV;
Human plasma

Abstract The aim of the research was to investigate the pharmacokinetics (PK) of enteric-coated mycophenolate sodium (EC-MPS) by quantification of the active metabolite of mycophenolic acid (MPA) after multiple escalating oral doses in Han kidney transplant recipients. A total of 28 Han postoperative kidney transplant recipients were given a multiple-dose of 540, 720 or 900 mg of EC-MPS two times a day in combination with tacrolimus for 6 days. Blood specimens were collected at each time point from 0 to 12 h after EC-MPS administration. MPA plasma concentrations were measured by UPLC–UV. The relationship between the EC-MPS dose and its PK parameters was assessed. In the range from 540 to 900 mg, C_{max} and AUC_{0-12h} did not increase with dose escalation. The AUC_{0-12h}, C_{max}, C_0 and T_{max} for the 540 720 and 900 mg doses were not significantly different, respectively ($P > 0.05$). $AUC_{0-12\,h}$ and C_{max} were increased less than proportionally with increasing EC-MPS dose levels. Inter-individual variability in AUC_{0-12h}, C_{max} and C_0 were considerable. Nonlinear PK relationships were found from the doses of 540–900 mg of EC-MPS.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.11.003

1. Introduction

The enteric-coated mycophenolate sodium (EC-MPS), a formulation of mycophenolic acid (MPA) is a standard immunosuppressive drug widely used in the renal transplant patients. Chemical structures of mycophenolate sodium and MPA are shown in Fig. 1. As compared with mycophenolate mofetil (MMF), EC-MPS shows the potential to reduce the incidence of adverse gastrointestinal effects by delaying MPA release until gastric emptying yet maintaining efficacy equivalent to that of MMF[1]. Although EC-MPS was used clinically as a fixed-dose drug, therapeutic drug monitoring (TDM) of the MPA area under the concentration–time curve (AUC) was found to improve clinical outcome[2-4]. MMF or EC-MPS, together with calcineurin inhibitors (either cyclosporine or tacrolimus) and steroids have become standard immunosuppressive therapy worldwide. A cyclosporine-based research project reported that treatment with 2724 mg of EC-MPS resulted in an AUC of MPA which was only 37% higher than treatment with 1440 mg of EC-MPS ten days after transplantation[5]. de Winter BC et al.[6] reported a nonlinear relationship between MMF dose and MPA AUC in renal transplant patients. There are limited PK data of EC-MPS in early Chinese kidney transplant recipients. A better understanding of the pharmacokinetic (PK) characteristics of EC-MPS in renal transplant recipients will improve the clinical effectiveness and safety profile of this medication. To increase our understanding of the PK characteristics of EC-MPS, we choose 3 dose groups in our research. The aim of the study was to test the PK behavior of EC-MPS over a range of 3 doses.

2. Materials and methods

2.1. Determination of plasma MPA concentrations

The method of UPLC–UV was used to analyze the MPA concentration in plasma. The validated UPLC–UV method was simple, accurate and successfully applied to the PK of EC-MPS study.

2.2. Chemicals

MPA, the reference standard (99.53% purity, Sigma, USA). EC-MPS was purchased from Novartis Pharma Schweiz AG. The internal standard of carbamazepine (IS) was purchased from the National Institute for Control of Pharmaceutical and Biological Products. Acetonitrile, which was HPLC-grade, was purchased from Merck Company, Inc. (Darmstaelt, Germany). Ultra-pure grade water was prepared using the Millipore Milli-Q purification system (Bedford, MA, USA). Hydrochloric acid, of HPLC-grade, was obtained from Tedia Company, Inc. (Fairfield, Ohio, OH, USA). Potassium dihydrogen phosphate is analytical pure.

2.3. Ultra performance liquid chromatography spectrometry

A UPLC-H-Class system (Waters Corporation) with Acquity UPLC and Acquity TUV detector (Waters Corporation), was used to determine the compounds. The samples were separated on an ACQUITY.UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm, Waters, USA); the mobile phase consisted of water (20 mmol/L potassium dihydrogen phosphate) and acetonitrile (69.5:30.5, *v/v*) at a flow rate of 0.25 mL/min; the UV detective wave length was 254 nm and column temperature was 35 °C.

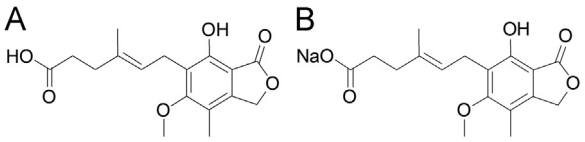

Figure 1 The chemical structures of (A) mycophenolic acid (MPA) and (B) mycophenolate sodium.

2.4. Sample preparation

Human plasma samples were thawed at room temperature. For each sample, an aliquot of 200 μL was added into 1.5 mL Eppendorf tubes with 400 μL IS (1 μg/mL) acetonitrile solution. After the tube was vortex mixed for 2 min, the mixture was centrifuged at 12,000 × *g* at 4 °C for 5 min, all of the supernatant was transferred into another Eppendorf tube and dried with nitrogen in a 40 °C water bath. Mobile phase (100 μL) was then added, followed by a thorough vortex mixing for 2 min, centrifuged at 12,000 × *g* at 4 °C for 3 min, and the upper layer (5 μL) was injected into the UPLC system.

2.5. Method validation

The validation of the UPLC–UV method for the determination of MPA in plasma samples was performed according to Food and Drug Administration (FDA) Guidelines. The lower limit of quantification (LLOQ), carryover effect, precision, accuracy, matrix effect, extraction recovery and stability tests were carried out to assess the method validation.

Specificity was evaluated by comparing the chromatograms of blank plasma from 6 different sources with the blank human plasma sample spiked with MPA and IS. Linearity of MPA was evaluated over the range of 0.10–40.00 μg/mL. The calibration curves were established by plotting the peak area ratio of MPA to IS *versus* the theoretical concentration of MPA, and fitted with by least square weighted linear regression. The sensitivity of the analytical procedure was expressed as LLOQ that can be quantitatively determined with acceptable accuracy and precision and should be with a signal–noise (*S/N*) ratio at least of 10.

The carryover effect was evaluated by injecting blank sample after the 40.00 μg/mL sample. Carryover in the blank sample following the 40.00 μg/mL sample should not be greater than 20% of the LLOQ for MPA and 5% for IS.

The matrix effects were determined by comparing the peak areas obtained from samples where the extracted matrix was spiked with standard solutions to those of the pure samples prepared in mobile phase containing equivalent amounts of the analyte in quality control (QC) samples. Recovery was calculated by comparing the peak area obtained from an extracted sample with that obtained from unextracted standard solution prepared with the same solvent.

Intra- and inter-day precision and accuracy were assessed by analysis of the QC samples. QCs at three levels (0.20, 5.00 and 32.00 μg/mL) were analyzed on 6 replicates during the same day and on 3 different days. The mean accuracy (%) was determined by comparing the measured concentrations against the theoretical concentration (mean concentrations/theoretical concentration × 100) for the QC samples.

The plasma samples and stock solutions stability tests were assessed at three QC levels in different conditions. Freeze and thaw stability were evaluated by four freeze–thaw cycles. Frozen samples were allowed to thaw at 25 °C for 12 h. Short-term stability was assessed by thawing at 25 °C and keeping at 25 °C