Journal of Pharmaceutical and Biomedical Analysis 131 (2016) 94–102

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Development and validation of an ultrafast chromatographic method for quantification of the immunosuppressant mycophenolic acid in canine, feline and human plasma

Sol-Maiam Rivera Vélez, Alice Morassi, Michael H. Court, Jennifer E. Slovak, Nicolas F. Villarino *

Program in Individualized Medicine, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman 99164, WA, United States

ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form 17 July 2016
Accepted 11 August 2016
Available online 12 August 2016

Keywords:
Mycophenolic acid
UHPLC
Dogs
Cats
Human

ABSTRACT

Mycophenolic acid (MPA) is the active metabolite of the prodrug mycophenolate mofetil. In this study, we developed and validated a novel ultra-high performance liquid chromatography (UHPLC) method for the rapid quantification of MPA in plasma from dogs, cats and humans. Following the protein precipitation, calibration standards and quality controls were separated by UHPLC reversed-phase on a 1.5 μm 2.1 × 100 mm C_{18} column and quantified using UV detection at 215 nm. The procedure produced a linear curve ($r^2 > 0.997$) over the concentration range 0.4–50 μg/mL and exhibited a high degree of repeatability (CV% <11%). The limit of detection (LOD) and lower limit of quantitation (LLOQ) were 0.1 and ≤0.4 μg/mL, respectively and the overall recovery was ≥87%. By combining isocratic conditions with a UHPLC column containing solid core particles, we were able to elute MPA and the internal standard (mycophenolic acid carboxybutoxy ether) within 3.0 min. The short total run time makes this method ideal to study the disposition of MPA in large batches of plasma samples and/or monitor plasma drug concentrations, as recommended for patients that require optimized immunosuppression.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Mycophenolic acid (MPA) is an active metabolite of the prodrug mycophenolate mofetil (MMF; Fig. 1). Mycophenolic acid inhibits inosine monophosphate dehydrogenase, preventing synthesis of lymphocyte DNA resulting in decreased lymphocyte proliferation, antibody production, cellular adhesion, and migration of T and B lymphocytes [1]. The MMF is extensively used in human medicine to prevent organ rejection and in the treatment of other immune-mediated conditions [2].

Following oral administration, MMF is hydrolyzed to MPA, N-(2-carboxymethyl)- morpholine, N-(2-hydroxyethyl)-morpholine and the N-oxide of N-(2-hydroxyethyl)-morpholine by carboxylesterases (CES)- 2 within the intestine [3]. The MMF that escapes initial intestinal hydrolysis enters into the liver via the portal vein and gets converted to MPA in the hepatocytes by CES, mainly CES-1 and CES-2 [3]. Mycophenolic acid is then metabolized into several metabolites, primarily the phenol and acyl glucuronides, by liver microsomal enzymes [4].

Due to the high safety and efficacy profiles of MMF reported in humans and the relative low cost of treatment, interest in the use of this drug in veterinary patients is growing. However, the disposition of MPA in canine and feline plasma and the optimal dosage regimens of MMF for use in these species remain unknown. In order to select safe and effective dosage regimes for dogs and cats, pharmacokinetic and pharmacodynamic studies are necessary. Pharmacokinetic studies require a species-specific, highly sensitive and reproducible analytical method. Multiple HPLC methods [5–14] and enzyme multiplied immunoassay technique (EMIT) [15–17] have been developed for the determination of MPA in human plasma. However, no analytical methods have been validated to date for the quantification of MPA in plasma from dogs and cats.

In this study, we developed and validated a novel ultra-high performance liquid chromatography (UHPLC) method that allowed for the rapid quantification of MPA in plasma from dogs, cats, and humans using protein precipitation, and a UHPLC column containing solid core particles and ultraviolet (UV) detection.

* Corresponding author.
E-mail address: nvillarino@vetmed.wsu.edu (N.F. Villarino).

http://dx.doi.org/10.1016/j.jpba.2016.08.012
0731-7085/© 2016 Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Reagents and chemicals

The standard MPA (purity, >98%) was purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). The internal standard (IS), mycophenolic acid carboxybutoxy ether (MPAC) (Fig. 1), was obtained from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). The compound MMF CellCept® intravenous was purchased from Roche Lab Inc. Methanol (MeOH), acetonitrile (ACN) and phosphoric acid (H_3PO_4) 85% were obtained from Fisher Scientific (Pittsburgh, PA, USA). Milli-Q water was used to prepare the mobile phase. Pooled untreated plasma collected in acid citrate dextrose from clinically healthy dogs (n = 6), cats (n = 6) and humans was used for assay validation.

2.2. Chromatographic conditions

UHPLC–UV analyses were performed on a Dionex Ultimate 3000 HPLC-system (Thermo Scientific, Waltham, MA), consisting of a degasser, a quaternary pump, an autosampler, a column thermostat, and an UV detector. Chromeleon 7 software from Dionex was used for data acquisition and processing. Separations were performed on an Accucore Vanquish UHPLC C_{18} column, (1.5 μm, 2.1 × 100 mm) (Thermo Scientific, Waltham, MA). The mobile phase consisted of ACN/0.05% H_3PO_4 in H_2O (50/50 v/v), pumped at a flow rate of 0.25 mL/min and UV detection at 215 nm. The column and sample temperatures were set at 40 and 25 °C, respectively. Injection volume was 1 μL.

2.3. Preparation of stock solutions and calibration standards

Stock solutions for MPA and MPAC were prepared in MeOH to give a final concentration of 1000 and 100 μg/mL, respectively. All solutions were stored at −80 °C. For preparation of calibration standards and quality control (QC) samples, plasma from each species was spiked with appropriate aliquots of MPA before the extraction procedure.

2.4. Sample preparation

A 200 μL aliquot of plasma (calibration standard or QC samples) was transferred into a 1.5-mL Eppendorf tube and 400 μL of 2 μg/mL IS in MeOH:ACN, 1:1, v/v was added. Each extract was then mixed for 30 s and centrifuged at 17,000g for 30 min. A 450 μL aliquot of supernatant was evaporated to dryness using a Speed-Vac concentrator (Savant Instruments Inc.). The residue was resuspended in 150 μL of MeOH: ACN: 0.5% H_3PO_4 in H_2O, (1:1:1, v/v/v), mixed, and centrifuged at 17,000g for 5 min to further sample cleanup. This second supernatant was injected into the UHPLC.

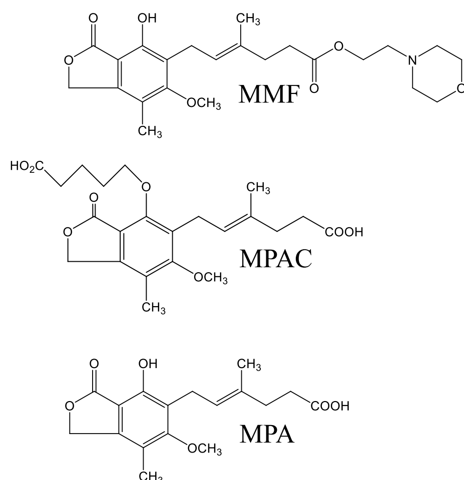

Fig. 1. Chemical structures of the prodrug, MMF; internal standard, MPAC; and the active metabolite, MPA. Chemical and physical properties of MPA includes: molecular weight: 320.34, log *P* = 3.88 for uncharged form, and pKa 4.5.

2.5. Plasma protein precipitation

In order to optimize the extraction of MPA from plasma we evaluated seven protein precipitation methods. Plasma proteins were precipitated using MeOH and/or ACN. Table 1 describes the precipitant agent and the volume ratio of precipitant to plasma used in each approach. The solvents, MeOH and ACN, were used as the precipitant agents because their effectiveness in precipitating plasma proteins is widely documented [18]. For *Method 2* the precipitant solution was acidified with 85% (v/v) H_3PO_4 to evaluate the following pHs: 3.0, 5.0 and 7.4. Each procedure was replicated three times. In order to select the optimal method we compared: (i) the extent of MPA recovery; (ii) the chromatographic profiles; and (iii) system pressure stability (pressure is mainly generated by the column). A single-factor analysis of variance (ANOVA) at a significance level of 5% was used to determine significant differences in MPA recovery between the protein precipitation methods. Microsoft Excel version 2010 (Microsoft Corp.) was used for data analysis.

2.6. Method validation

The method was validated according to the Guidelines for Bioanalytical Method Validation published by the Food and Drug Administration (FDA) [19]. Validation of the method was carried out using QC samples. All the QC samples and calibration standards were prepared in species-specific plasma. The concentration of the QCs used to determine accuracy, recovery and short term stability were 1.0, 12.5 and 50 μg/mL while intra- and inter-day precisions were evaluated at 0.5, 1.0, 1.6, 12.5, 25 and 50 μg/mL.

2.6.1. Calibration curve

The linearity was investigated by calculation of the regression curve by the method of least squares and expressed as the coefficient of determination (R^2). The range of concentrations included in the calibration curve (from 0.4 to 50 μg/mL) was defined based on the range of plasma MPA concentration reported in dogs during a dose interval (12 h) after a therapeutic dose (20 mg/kg orally; 9.3–24.8 μg/mL) [20].

2.6.2. Intra and inter-day precision

Intra and inter-day precisions were assessed by analysis of spiked QC samples by sextuplicate during the same day and on three different days. The precision was determined by calculating the coefficient of variation (CV%) [19].

2.6.3. Accuracy

The accuracy, expressed as relative error (%Er), was determined by comparing the measured concentration against the theoretical concentration ((mean of measured concentration −theoretical concentration)/theoretical concentration × 100) [19,21].

2.6.4. Lower limit of quantitation (LLOQ), limit of detection (LOD) and upper limit of quantification (ULOQ)

The LOD and LLOQ were calculated using the equations LOD = 3.3Sb/a and LLOQ = 10Sb/a, where a is the curve slope and Sb is the standard deviation (SD) of the intercept [19,22]. The ULOQ was the highest concentration in the standard curve for which both

Download English Version:

https://daneshyari.com/en/article/1220124

Download Persian Version:

https://daneshyari.com/article/1220124

Daneshyari.com