International Journal of Pharmaceutics 441 (2013) 19–29

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Nebulization of mycophenolate mofetil inhalation suspension in rats: Comparison with oral and pulmonary administration of Cellcept®

Hélène L. Dugas[a,*], Jay I. Peters[b], Robert O. Williams III[a,*]

[a] College of Pharmacy, Pharmaceutics Division, The University of Texas at Austin, TX, USA
[b] Department of Medicine, Division of Pulmonary Diseases/Critical Care Medicine, The University of Texas Health Science Center at San Antonio, TX, USA

ARTICLE INFO

Article history:
Received 17 May 2012
Received in revised form
30 November 2012
Accepted 12 December 2012
Available online 20 December 2012

Keywords:
Mycophenolate mofetil
Immunosuppressant
Pulmonary delivery
Preclinical pharmacokinetics
Lung
Lymphatic system

ABSTRACT

In this study, a suspension of mycophenolate mofetil (MMF) suitable for inhalation was developed using the emulsion template process and characterized for particle size and aerosolization performance. To evaluate the benefits of this suspension over a solution, the IV Cellcept® solution was also characterized *in vitro*. Both formulations exhibited excellent aerosolization performance. The aerodynamic diameters for the solution and the suspension were within the respirable range (below 5 μm) and their fine particle doses were nearly equivalent, suggesting the same drug exposure during *in vivo* experiments. Single dose 24-h pharmacokinetic studies following inhalation of the formulations and oral administration of oral Cellcept® were performed in rats. Following oral administration, MMF was completely and rapidly metabolized into its active metabolite, mycophenolic acid (MPA) and partial metabolism was observed following pulmonary administration. Inhaled MMF suspension displayed more favorable pharmacokinetics than inhaled IV Cellcept® solution, but the MPA drug levels in each compartment were much lower than those obtained with oral Cellcept®. The dose normalized MPA levels in the lung, thymus gland and plasma following inhalation of the MMF suspension with the oral control suggested that pulmonary delivery of a MMF suspension could be beneficial in preventing lung allograft rejection.

1. Introduction

Lung transplantation is a life-saving intervention for patients suffering from end-stage pulmonary diseases. Currently, however, the median survival is only 5.5 years (Christie et al., 2011). Beyond the first year after lung transplantation, graft failure, non-cytomegalovirus infections and bronchiolitis obliterans syndrome are the leading cause of death (Benden et al., 2012), suggesting that improvements in the long-term management of acute and chronic rejection are necessary.

Mycophenolate mofetil (MMF) is the ester prodrug of mycophenolic acid (MPA), an anti-metabolite immunosupressant. MPA is a non-competitive inhibitor of the *de novo* purine biosynthesis of guanosine nucleotides necessary for the production of activated T- and B-lymphocytes (Stepkowski, 2000). MPA has proven its efficacy among transplanted patients (Bardsley-Elliot et al., 1999). Upon absorption, MMF is rapidly hydrolyzed into MPA by the carboxylesterases present in the intestine wall and in the liver (Fujiyama et al., 2010). Due to its rapid excretion, a high daily oral dose (up to 3 g/day) is necessary, leading to GI tract toxicity and myelosuppression (Parfitt et al., 2008; Ting et al., 2006). These side effects often force the patients to decrease their daily dose and even sometimes to stop the treatment, increasing the risks of allograft rejection (Kaushik et al., 2006). Therefore, developing new therapeutic strategies for the delivery of MMF is critical to improve patient outcomes.

Pulmonary therapy offers an interesting approach to drug delivery thanks to its large area of absorption and high vascularization, its capability to avoid the hepatic first-pass metabolism, and its ability to achieve high lung deposition and reduced systemic drug concentration. This noninvasive route of administration has the potential to minimize toxicity without compromising efficacy and therefore improves patient compliance. The inhalation route has already been used widely in the treatment of respiratory diseases (Carvalho et al., 2011; Dailey, 2007; Iacono et al., 2006; Tolman and Williams, 2010). Consequently, the pulmonary delivery of MMF could benefit lung transplant patients by decreasing the systemic side effects.

Particle engineering techniques for pulmonary drug delivery have significantly improved in the recent years, enabling more efficient drug deposition to the lungs (Chow et al., 2007; Patravale

* Corresponding authors at: College of Pharmacy, Pharmaceutics Division, The University of Texas at Austin, 2409 West University Avenue, Mail Stop A1920, Austin 78712, TX, USA. Tel.: +1 512 471 4681; fax: +1 512 471 7474.
E-mail addresses: hdugas@utexas.edu (H.L. Dugas), williro@mail.utexas.edu (R.O. Williams III).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.12.016

et al., 2004). The pulmonary delivery of microparticles allows the drug to reach the deep lung when their aerodynamic diameter is less than about 5 μm. Besides, microparticles offer the advantage of providing prolonged drug release (El-Sherbiny et al., 2011) and promoting alveolar macrophages uptake (Ahsan et al., 2002; Makino et al., 2004; Sharma et al., 2001), when their size is between 0.5 μm and 3 μm (Kreyling and Scheuch, 2000; Makino et al., 2003). In the case of an immunosuppressive therapy for lung transplanted patients, since the actors of the immune response are present in the transplanted lung as well as in the lymphatic system, it is important to achieve simultaneously high immunosuppressant levels in the lungs and in the lymphatic system. Therefore, the pulmonary administration of microparticles could deliver MMF to the lungs and the lymphatic tissues *via* alveolar macrophages uptake.

A recent study in our laboratory has demonstrated the ability of human lung cells to hydrolyze MMF *in vitro* into it active metabolite, MPA. This work hypothesized that a micron size MMF suspension, with particles within the 1–2 μm range, can be developed and achieve high and sustained drug levels in the lung and the lymphoid tissues while keeping a low systemic concentration following inhalation. To the best of our knowledge, tissue distribution of MPA after oral administration of oral Cellcept®, the currently commercialized product, has not been reported yet, hence this study reports the distribution of MPA in the lung, thymus gland and plasma following administration of oral Cellcept® by mouth. A micron size MMF suspension containing particles within the 1–2 μm range was developed using particle engineering techniques. Its *in vitro* physicochemical and aerodynamic properties were evaluated. Finally, the pharmacokinetic profile and systemic bioavailability of MMF after pulmonary administration of the micron size MMF suspension were investigated in rats and compared to the profiles obtained after nebulization of the IV Cellcept® solution and oral administration of oral Cellcept®.

2. Materials and methods

2.1. Chemicals and reagents

Mycophenolate mofetil and mycophenolic acid were purchased from Trademax Pharmaceuticals & Chemicals Co., Ltd (China). Mycophenolate mofetil reference standard was bought from USP (Rockville, MD) and mycophenolic acid reference standard from Spectrum (Gardena, CA). Acetonitrile (ACN) for HPLC, triethylamine (TEA) for HPLC, ethanol absolute (200 proof) molecular biology grade, methylene chloride stabilized and certified ACS, spectranalyzed methanol and ortho-phosphoric acid for HPLC were obtained from Fisher Scientific (Pittsburgh, PA). Potassium phosphate monobasic, indomethacin (the internal standard, IS) and tyloxapol were purchased from Spectrum (Gardena, CA). 1,2 dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Genzyme Pharmaceuticals (Cambridge, MA). All other chemicals and solvents were of the highest grade commercially available.

2.2. Formulation preparation

2.2.1. Cellcept® suspension

The Cellcept® suspension for oral gavage was prepared as follows. Nine 250-mg Cellcept® capsules, containing 250 mg of the active pharmaceutical ingredient with 50 mg of excipients (croscarmellose sodium, magnesium stearate, povidone (K-90) and pregelatinized starch), were opened and poured into 30 ml of 0.1% (w:v) xanthan gum solution. The suspension was probe sonicated for 10 min at 50% duty cycle and 5 output control, to uniformly disperse the powder in solution. To rinse the sonication probe and reach a final MMF concentration of 50 mg/ml, 15 ml of 0.1% (w:v) xanthan gum solution was added. The xanthan gum was used to prevent particle aggregation prior to administration. The final product will be referred hereafter as oral Cellcept® suspension.

2.2.2. IV Cellcept® solution

The IV Cellcept® solution contained the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt with 25 mg polysorbate 80 and 5 mg citric acid. The solution was prepared by dissolving the IV Cellcept® preparation in a 5% dextrose solution for injection (D5W) as indicated in the package insert. Subsequent adequate dilutions with D5W were performed to obtain a 25 mg/ml solution referred hereafter as IV Cellcept® solution.

2.2.3. Engineered particles of mycophenolate mofetil in suspension

The micron-size MMF suspension with particles ranging from 1 to 2 μm was prepared using the emulsion template process. Briefly, DPPC and MMF were dissolved in 1 ml of ethanol and 6 ml of dichloromethane. This solution was added dropwise to 50 ml 0.2 g/L tyloxapol in D5W solution while sonicating with an analog Branson Sonifier® cell disruptor/homogenizer 450 fitted with a ½″ diameter tapped bio horn at 50% duty cycle and 5 output control and cooled down in an ice bath. After addition of the DPPC/MMF solution, the sonication was continued for 5 min. The solvents were extracted at 27 °C for 10 min by vacuum evaporation using a Buchi® rotary evaporator system consisting of a distillation chiller B-741, a vacuum pump V-700 and a rotavap R-210. The duration of the evaporation was determined by witnessing two boiling events corresponding to the evaporation of dichloromethane and ethanol, respectively. The evaporation was pursued 5 min after the end of the second boiling event to ensure solvent removal; however, residual solvents were not determined quantitatively in the final formulation. Due to the azeotropic nature of the solvent mixture, the volume of the final suspension was readjusted to 50 ml with the 0.2 g/L tyloxapol in D5 W solution and further sonicated for 5 min at 50% duty cycle and 5 output control in an ice bath. The final suspension pH was adjusted to about 7 by the addition of an appropriate volume of 0.5 N sodium hydroxide solution (~40 μl) and left at room temperature to equilibrate before characterization. The final concentration the micron-size MMF suspension was 25 mg/ml and will be referred hereafter as the MMF suspension.

2.3. Formulation characterization

2.3.1. Particle size analysis

The particle size analysis was determined by Dynamic Light Scattering (DLS) using the Malvern Zetasizer Nano S® (Malvern Instrument Ltd, Worcestershire, UK). The measurements were performed at 25 °C with a pre-measurement equilibration time of 1 min. The samples were diluted with 0.2 g/L tyloxapol in D5 W to have the intercept of the correlation function between 0.7 and 1. The refractive indexes for the dispersant (water) and the internal phase (phospholipids) were set at 1.33 and 1.45 respectively. The *z*-average size and the polydispersity index (PdI) were recorded for each formulation and the measurements were done in triplicate.

2.3.2. Morphology

Scanning electron microscopy (SEM) was used to examine the surface morphology of the MMF particles present in the suspension. The suspension was quickly frozen in liquid nitrogen and lyophilized using a Virtis Advantage 2.0 BenchTop freeze dryer (SP Scientific, Warminster, PA) to obtain a dry powder. Samples were loaded onto double-sided carbon tape and sputter coated with a 60/40 Pd/Au target for 4 min. SEM images were captured using a