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Research paper

Characterization and pharmacokinetic analysis of crystalline versus amorphous rapamycin dry powder via pulmonary administration in rats

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ABSTRACT

The pharmacokinetics of inhaled rapamycin (RAPA) is compared for amorphous versus crystalline dry powder formulations. The amorphous formulation of RAPA and lactose (RapaLac) was prepared by thin film freezing (TFF) using lactose as the stabilizing agent in the weight ratio 1:1. The crystalline formulation was prepared by wet ball milling RAPA and lactose and posteriorly blending the mixture with coarse lactose (micronized RAPA/micronized lactose/coarse lactose = 0.5:0.5:19). While both powders presented good aerosolization performance for lung delivery, TFF formulation exhibited better *in vitro* aerodynamic properties than the crystalline physical mixture. Single-dose 24 h pharmacokinetic studies were conducted in Sprague–Dawley rats following inhalation of the aerosol mist in a nose-only inhalation exposure system. Lung deposition was higher for the crystalline group than for the TFF group. Despite higher pulmonary levels of drug that were found for the crystalline group, the systemic circulation (AUC_{0-24}) was higher for the amorphous group (8.6 ng h/mL) than for crystalline group (2.4 ng h/mL) based on a five-compartmental analysis. Lung level profiles suggest that TTF powder stays in the lung for the same period of time as the crystalline powder but it presented higher *in vivo* systemic bioavailability due to its enhanced solubility, faster dissolution rate and increased FPF at a more distal part of the lungs.

1. Introduction

Rapamycin (Sirolimus, RAPA) is a carboxylic lactone–lactam macrolide antibiotic with antifungal activity and great immunosuppressive and antitumor properties [1]. RAPA has been used both with and without cyclosporine in order to prevent organ rejection in patients after kidney transplant and as an alternative immunosuppressant for lung transplantation [2]. Numerous noted side effects, such as hypertension, hyperlipidemia and nephrotoxicity, have impeded its use in treatment for a prolonged time [3]. Recently, systemic RAPA has been investigated for treating women diagnosed with pulmonary involvement with lymphangioleiomyomatosis (LAM). LAM is a progressive, cystic lung disease associated with inappropriate activation of mammalian target of rapamycin (mTOR) signaling, which regulates cellular growth and lymphangiogenesis. In 2011, the Multicenter International LAM Efficacy of Sirolimus (MILES) Trial was performed. This double-blind, placebo-controlled trial showed promising results in phases 1 and 2, where oral doses of RAPA successfully inhibited mTOR and stabilized lung function in patients with moderate to severe LAM [4,5]. Unfortunately, the disease progressed when rapamycin was discontinued and the systemic toxicities were felt to be too significant for long-term use in young females.

RAPA is currently approved for prophylaxis of rejection for kidney allograft patients and it is only commercially available for oral administration (e.g. Rapamune®) due to its poor water solubility (2.6 μg/mL) [6]. The oral bioavailability of RAPA from Rapamune® oral solution is approximately 14% [7,8]. The oral bioavailability was reportedly improved to about 27% reducing the particle size [9,10]. Therefore, because of the relatively low oral bioavailability, a higher dose of RAPA is given to the patient in order to reach the desired drug therapeutic window [7,11]. Such high dosages may be the cause of various adverse effects. To further improve RAPA

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solubility, numerous oral formulations have been reported e.g. solid dispersion nanoparticles using a supercritical antisolvent process [12], liposomes [13] and solid dispersion and complexation with hydrophilic excipients [14].

To our knowledge, rapamycin has never been used to treat systemic or local diseases via pulmonary inhalation. Pulmonary drug delivery is a non-invasive route of administration which presents several advantages such as avoidance of first pass metabolism and large mucosal surface area for drug absorption; a characteristic which allows this method to be an ideal candidate in applications involving local and systemic administration [15].

Polymorphic and amorphous forms of poorly water-soluble drugs have been developed to enhance local and/or systemic bioavailability when delivered via the pulmonary route. Wei et al. compared the bioavailability of amorphous versus crystalline itraconazole nanoparticles via pulmonary administration in rats. After inhalation of the same dose of nebulized itraconazole dispersions, the maximum concentration (C_{max}) of the nanocrystalline dispersion was 50 ng/mL at 2.7 h with an AUC_{0-24} of 662 ng h/mL. The C_{max} of amorphous dispersion was 180 ng/mL at 4 h and AUC_{0-24} of 2543 ng h/mL, in systemic circulation. The authors relate the significantly higher systemic bioavailability value of the amorphous system to the increased supersaturated environment, which would result in an increased drug permeation [16]. Despite these findings, in another study, the administration of amorphous voriconazole nanoparticles to the lungs of mice did not present enhanced systemic bioavailability when compared to the crystalline counterpart. Beinborn et al. associate these results to the prolonged residence time of crystalline voriconazole in the lungs when compared to the amorphous system [17].

The aim of this study was to assess and compare the *in vivo* behavior and pharmacokinetic profiles of crystalline and amorphous rapamycin when administered to the lungs of rats via dry powder inhalation. We hypothesize that the solubility enhancement of amorphous rapamycin in the lung fluids will increase *in vivo* systemic bioavailability. Techniques used in this study include thin film freezing technology (TFF) in order to produce amorphous rapamycin respirable powder and wet ball milling to prepare the alternative crystalline rapamycin powder.

2. Materials and methods

2.1. Materials

Rapamycin (Sirolimus) was purchased from Tecoland Corporation (Irvine, California) and lactose monohydrate (lactose, Lactohale® LH 200) was kindly donated by Friesland Foods Domo (Zwolle, Netherlands). High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Water was purified by reverse osmosis (MilliQ, Millipore, France).

2.2. Formulation preparation

Thin film freezing technology was used for the preparation of amorphous and low density dry powder [18]. In brief, a cosolvent mixture of acetonitrile and water (3:2) was used to dissolve rapamycin and lactose in the ratio of 1 to 1. To investigate the influence of solid loading on TFF powder aerosolization, two formulations were prepared with different final solid loading concentrations: 0.40% and 0.75% (w/v). The solution was rapidly frozen on a cryogenically cooled (−80 °C) stainless steel surface by thin film freezing. The frozen films were collected in a container filled with liquid nitrogen to avoid melting. The frozen formulation was transferred to a −70 °C freezer until the liquid nitrogen was completely evaporated, and then transferred to a VirTis Advantage Lyophilizer (VirTis Company Inc., Gardiner, NY) for solvent removal. Formulation was lyophilized over 24 h at −40 °C at a pressure of 400 mTorr. The temperature was gradually increased to 25 °C over 24 h with a pressure less than 200 mTorr, and kept at 25 °C for 24 h.

For comparison purposes, the equivalent crystalline physical mixture of rapamycin and lactose in the same weight ratio as used with the TFF powder formulations was prepared. First, micronized rapamycin and lactose were prepared using wet ball milling in a ceramic jar with zirconia grinding media (1/2″ radius end cylinder) (US Stoneware, East Palestine, OH). Five grams of rapamycin powder was added to 25 mL of purified water and milled at 100 rpm at 20 °C for 48 h. Likewise, lactose was dispersed in acetonitrile and ball milled at 100 rpm at 20 °C for 48 h. The obtained slurry collected from the ceramic jar and from triple rinsing of milling media was frozen at −80 °C and then lyophilized using a VirTis Advantage Lyophilizer (VirTis Company Inc., Gardiner, NY) for solvent removal. The dry powder products were stored in a desiccator under vacuum at room temperature until further use. After particle size reduction, lactose monohydrate and rapamycin were sieved through a 100 μm and 45 μm mesh. Equivalent amounts of micronized lactose and rapamycin were accurately weighed and mixed using the geometric dilution technique. The mixed powder was then mixed with coarse lactose LH200 in the ratio 1 to 19, again using geometric dilution technique. The final mixture was then transferred to a stainless steel mixing vessel. The vessel was placed in a Turbula Blender T2F (Bachofen, Switzerland) and mixing was carried out for 20 min at 48 rpm.

2.3. Particle size analysis

Measurements of particle size distributions of rapamycin and lactose, before and after wet ball milling, were taken by laser diffraction (HELOS, Sympatec GmbH, Clausthal-Zellerfelg, Germany). A small amount of bulk lactose was dispersed in 10 mL 0.01% Tween 80 mineral oil and a small amount of rapamycin was dispersed in 10 mL 0.01% Tween 80 in deionized water. The samples were sonicated for 5 min and diluted with enough solvent to produce light obscuration in the range of 10–20%. Results are presented as $D_{(X)}$ and span, where X is the cumulative percentile of particles under the referred size (e.g. $D_{(50)}$ corresponds to the median diameter of the particles). Span is a measurement of particle size distribution calculated as $[(D_{(90)} - D_{(10)}]/D_{(50)}$. The sizes reported are average values taken from at least 3 measurements.

2.4. Scanning Electron Microscopy (SEM)

Analysis of powder morphologies and estimation of particle size of all samples were performed using SEM. Samples were placed on carbon tape and coated with gold/palladium (60/40) for 20 s under argon atmosphere using a Cressington Sputter Coater 208 HR (Cressington Scientific Instruments, Watford, England). The SEM images were captured using a SmartSEM® graphical user interface software in a Carl Zeiss Supra® 40VP (Carl Zeiss, Oberkochen, Germany) operated under vacuum, at a working distance of 19 mm and at 5 kV of Electron High Tension (EHT).

2.5. Brunauer–Emmet–Teller (BET) specific surface area (SSA)

Powder porosity was determined through the measurement of the specific surface area (SSA) using a Monosorb MS-22 rapid surface area analyzer (Quantachrome Instruments, Boynton Beach, Florida). The instrument uses a modified BET equation for SSA determination. Samples were degassed in a Thermoflow™ Degasser for at least 2 h at 25 °C using 30% nitrogen in helium as the desorbate gas.