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Research paper

Nebulization of nanoparticulate amorphous or crystalline tacrolimus – Single-dose pharmacokinetics study in mice

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Abstract

Developing a pulmonary composition of tacrolimus (TAC) provides direct access to the graft in lung transplant offering the possibility of high drug levels. The objective of this study was to investigate the physicochemical and pharmacokinetic characteristics of the nanostructured aggregates containing amorphous or crystalline nanoparticles of TAC produced by ultra-rapid freezing (URF). TAC and lactose (1:1 ratio; URF-TAC:LAC) and TAC alone (URF-TAC) were investigated for pulmonary delivery and compared to unprocessed TAC. X-ray diffraction (XRD) results indicated that URF-TAC was crystalline, whereas URF-TAC:LAC was amorphous. *In vitro* results revealed the superior physiochemical characteristics of both URF formulations compared to unprocessed TAC. The surface area of URF processed TAC was higher (25–29 m^2/g) than that of the unprocessed TAC (0.53 m^2/g) and subsequently enhanced dissolution rates. In addition, URF-TAC:LAC displayed the ability to supersaturate in the dissolution media to about 11 times the crystalline equilibrium solubility. Similar aerodynamic particle sizes of 2–3 μm, and fine particle fraction between 70% and 75% were found in both formulations. The local and systemic pharmacokinetic studies in mice showed similar $AUC_{(0-24)}$, higher C_{max}, and lower T_{max} for the URF-TAC:LAC compared to the URF-TAC. Nanostructured aggregates containing amorphous or crystalline nanoparticles of TAC were demonstrated to be effectively delivered via nebulization, with similar *in vitro* and *in vivo* performances.

Keywords: Tacrolimus (TAC); Ultra-rapid freezing (URF); Nanoparticles; Amorphous; Crystalline; Pulmonary delivery

1. Introduction

Tacrolimus (TAC) is a widely used immunosuppressive agent isolated from *Streptomyces tsukubaensis*. It has proven to be a potent immunosuppressant in transplantation medicine for treatment of organ rejection and different immunological diseases such as pulmonary fibrosis and bronchiolar asthma [1–3]. TAC was first introduced as rescue therapy when cyclosporin A (CsA) therapy failed to prevent graft rejection [4]. It has a mechanism of action similar to that of CsA, but its immunosuppressive activity is 10 to 100 times more potent than CsA [5]. TAC is currently available in both an intravenous and an oral dosage form (commercially known as Prograf®). However, these current available dosage forms of the drug are poorly tolerated and provide a variable and/or low bioavailability [6]. The oral formulations of TAC present a considerable challenge as the drugs are practically insoluble in water and extensively metabolized from both CYP3A4 metabolism and p-glycoprotein efflux transport within the intestinal epithelium [7]. The oral bioavailability of TAC varies from 4% to 93% [8]. Inefficient or erratic drug absorption is primarily the result of incomplete absorption from the

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gastrointestinal tract and first-pass metabolism, which is subject to considerable inter-individual variation [8].

This study focused on investigating a first pulmonary drug delivery system based on nanoparticles of TAC in order to overcome the above-mentioned problems to improve bioavailability. The appealing aspects of inhaled drug nanoparticles include rapid dissolution of nanoparticles in the lung and the avoidance of hepatic first-pass metabolism (which is especially useful for a drug that undergoes extensive metabolism in liver) [9–11]. Additionally, inhaled nanoparticles can increase local drug concentrations in the lung for potential therapeutic use in lung transplantation and pulmonary diseases. The treatment of lung transplant recipients is often limited due to poor penetration of drug into the lung following oral or intravenous administration [12]. Aerosolized drug will have direct access to the graft in lung transplant offering the possibility of much higher drug levels [13,14]. However, a major disadvantage of pulmonary delivery for drugs like TAC is limitations in the levels and types of excipients that are considered safe to use in pulmonary formulations. Lactose is the most commonly used as the excipient for pulmonary delivery since it is approved by the Food and Drug Administration (FDA) as excipient for inhalation purposes [15]. This is due to their non-toxic, its broad availability, relatively low price and readily degradable properties after administration. In addition, lactose can inhibit crystallization of drug in amorphous phase and prevent nanoparticle aggregation upon lyophilization leading to a high dissolution of drug product [16].

Although many surfactants or polymers such as cyclodextrins, poloxamers, polyethylene glycols (PEG) and glycerol have been studied in pulmonary formulations to aid drug solubilization in many research studies [17–19], these excipients have not been approved yet for commercial use by the FDA because of potential toxicity in the lung. Several clinical studies have demonstrated effective pulmonary delivery of CsA solutions in ethanol or propylene glycol prior to aerosolization in lung transplantation models [20–22]. However, the solvents that have produced the results have shown to be unsatisfactory due to the irritating properties of the solventsation to the airways. In addition, the use of high levels of ethanol or propylene glycol in formulations intended for pulmonary delivery has yet to be widely studied in humans. Recently, liposome technology has been investigated as a non-irritating alternative for pulmonary delivery of CsA, but the formulation had low drug loading and thus requires a lengthy nebulization period [23].

In this present study, pulmonary formulations containing TAC manufactured by ultra-rapid freezing (URF), without the inclusion of surfactants or polymeric excipients, were investigated. URF technology is a continuous, scalable cryogenic process produces nanostructured aggregates with high surface area resulting in high enhanced drug dissolution rates. Previously, spray freezing into liquid (SFL) was reported [24–29]. The rapid freezing rates achieved with the SFL process led to the production of amorphous nanostructured aggregates composed of primary particles, ranging from 100 to 200 nm, with high surface areas, high wettability and significantly enhanced dissolution rates. The URF process yields particles with similar properties as those produced by SFL. In URF a solution of the active and excipient in a suitable organic solvent or aqueous co-solvent is applied to the surface of a cryogenic solid substrate. The solution is frozen very quickly in 50 ms to 1 s, onto the surface of cryogenic solid substrate in a continuous manner [30,31]. URF powders exhibit desirable properties for enhancing bioavailability such as high surface area, increased drug dissolution rates, and amorphous character.

The objective of this study was to demonstrate that nanostructured aggregates composed of amorphous or crystalline nanoparticles of TAC produced by the URF process are suitable for pulmonary delivery by nebulization, resulting in high lung and blood concentrations. The hypothesis is that high surface area and rapid dissolution rate obtained from nanostructured aggregates of TAC promote high systemic drug absorption via the lung, while still maintaining a desirable pulmonary residence time for potential local therapy.

Relevant physicochemical properties (e.g. surface area, dissolution, morphology and crystallinity) of TAC nanostructured aggregates were characterized in order to understand how they influence drug deposition and absorption following single-dose nebulization. The aerosol performance and deposition of TAC were determined using an 8-stage cascade impactor after aerosolization via an Aeroneb®. *In vivo* studies were conducted in mice by dispersing the URF formulations in deionized water and nebulizing the dispersed URF formulations using a specially designed nose-only dosing apparatus. Determination of tissue and serum concentrations was performed to characterize pharmacokinetic parameters for TAC.

2. Materials and methods

2.1. Materials

TAC was kindly provided by The Dow Chemical Company (Midland, MI). Anhydrous lactose, magnesium chloride hexahydrate, sodium chloride, potassium chloride, sodium phosphate dibasic anhydrous, sodium sulfate anhydrous, calcium chloride dihydrate, sodium acetate trihydrate, sodium bicarbonate and sodium citrate dihydrate were of analytical grade and purchased from Spectrum Chemicals (Gardena, CA). Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma–Aldrich Chemicals (Milwaukee, WI). High performance liquid chromatography (HPLC) grade acetonitrile (ACN) was purchased from EM Industries, Inc. (Gibbstown, NJ). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ). Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France).