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Research Paper

Pulmonary delivery of pyrazinamide-loaded large porous particles

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ABSTRACT

We have improved the aerodynamic properties of pyrazinamide loaded large porous particles (PZA-LPPs) designed for pulmonary delivery. To overcome the segregation of the different components occurring during the spray drying process and to obtain homogeneous LPPs, spray drying parameters were modified to decrease the drying speed. As a result, good aerodynamic properties for lung delivery were obtained with a fine particle fraction (FPF) of 40.1 ± 1.0%, an alveolar fraction (AF) of 29.6 ± 3.1%, a mass median aerodynamic diameter ($MMAD_{aer}$) of 4.1 ± 0.2 μm and a geometric standard deviation (GSD) of 2.16 ± 0.16. Plasma and epithelial lining fluid (ELF) concentrations of pyrazinamide were evaluated after intratracheal insufflation of PZA-LPPs (4.22 mg kg^{-1}) into rats and compared to intravenous administration (iv) of a pyrazinamide solution (5.82 mg kg^{-1}). The *in vivo* pharmacokinetic evaluation of PZA-LPPs in rats reveals that intratracheal insufflation of PZA-LPPs leads to a rapid absorption in plasma with an absolute bioavailability of 66%. This proves that PZA-LPPs dissolve fast upon deposition and that PZA crosses efficiently the lung barrier to reach the systemic circulation. PZA concentrations were 1.28-fold higher in ELF after intratracheal administration than after *iv* administration and the ratio of ELF concentrations over plasma concentrations was 2-fold greater. Although these improvements are moderate, lung delivery of PZA appears an interesting alternative to oral delivery of the molecule and should now be tested in an infected animal model to evaluate its efficacy against *Mycobacterium tuberculosis*.

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1. Introduction

Tuberculosis (TB) is an infectious disease due to *Mycobacterium tuberculosis*, affecting mainly the lungs [1]. TB current treatment, as recommended by the WHO, consists of a combination of orally-administered four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide [2–4]. Despite the efficacy of the TB regimen, the emergence of drug-resistant strains of *M. tuberculosis* and the lack of new anti-TB drugs represent a threat for the containment of TB epidemy. Two problems are responsible for the emergence of drug-resistance: some patients do not comply with the long treatments required and mycobacteria may be exposed to sub-therapeutic levels of antibiotics [5,6]. In patients, numerous bacteria populate lung lesions leading to the formation of granulomas. Due to the characteristics of these granulomas, conventional oral and parenteral therapies are inefficient in providing therapeutic levels of anti-TB drugs to bacteria sequestered therein. Among therapeutic molecules, pyrazinamide (PZA) is particularly interesting as it is active against a population of non-growing, persistent tubercle bacilli that other TB drugs cannot destroy [7–9]. These bacilli are present in the above-described lung lesions. The fraction of PZA reaching these lesions after oral administration therefore helps to shorten TB treatment.

Pulmonary delivery of anti-tubercular drugs has been proposed as an alternative strategy to obtain higher drug concentrations close to the granulomatous lesions [10,11]. Since the lungs are directly targeted, total body doses are lower, leading to a decrease of potential drug resistance buildup. This is of particular interest

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for PZA to favor high drug concentration in the vicinity of sequestered bacteria and facilitate their eradication.

Anti-TB drugs can be delivered to the lungs by nebulization [12–14] or as dry powders for inhalation [15–18]. Among dry powders for inhalation, large porous particles (LPPs) have emerged since the end of the 90s for both local and systemic treatments due to their efficient deposition in the lungs using simple inhalation devices [19–22]. By taking advantage of the LPP technology, TB treatment could be more efficient since antibiotics would be delivered directly to the site of infection to yield therapeutic local drug concentrations with lower systemic exposure than oral delivery [10].

PZA-LPPs were previously optimized by spray drying using excipients preventing PZA recrystallization and promoting particle stability [23]. Herein, we present the *in vitro* characterization of the aerodynamic properties of PZA-LPPs. PZA-LPPs were also administered to rats by insufflations and a thorough evaluation of the drug pharmacokinetics and broncho-alveolar lavage content was performed and compared with an iv administration of PZA through a pharmacokinetic model.

2. Materials and methods

2.1. Materials

Pyrazinamide (PZA) was obtained from Fluka, with specified purity greater than 99%. 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was provided by Corden Pharma (Switzerland) and hyaluronic acid, sodium salt 95% (MW = 1000 kDa) by Acros organics. Ammonium bicarbonate, acetazolamide and DL-Leucin were provided by Sigma–Aldrich (France). Ethanol absolute in analytical grade was obtained from Carlo Erba Reagents (France). Water was purified using a RIOS/MilliQ system from Millipore (France). HPLC-grade acetonitrile and methanol were purchased from Prolabo (France).

2.2. Particle formulation via spray drying

Large porous particles were obtained by spray drying using a mini spray dryer Büchi B-290 (Flawil, Switzerland) equipped with a 0.7 mm diameter two-fluid nozzle, which operates in a co-current mode. The formulation chosen was previously optimized in terms of composition [23] to prevent PZA recrystallization and yield stable large porous particles. The spray drying parameters such as air-flow rate, feed-flow rate, inlet temperature and aspiration are reported in Table 1. Briefly, DPPC was dissolved into 700 mL ethanol, whereas PZA and Leu were dissolved into 300 mL water. Then, hyaluronic acid was added into the aqueous solution and stirred using a magnetic stir bar for about an hour until dissolution. Afterward, ammonium bicarbonate was dissolved into the aqueous solution and subsequently ethanolic and aqueous solutions were mixed immediately prior to atomization. The final concentration of ammonium bicarbonate in the ethanol/water mixture was 2 g/L. The final solid content of the solution was 2 g/L omitting ammonium bicarbonate since this compound decomposes into water and gas during the drying process. Powder samples were stored at room temperature under vacuum in a desiccator immediately after spray drying to limit moisture uptake by samples between production and testing. The yield was calculated as a percentage by dividing the mass of the powder collected by the initial mass of solids in the solution prior to spray drying.

Table 1
Operational conditions used for spray drying the initial optimized formulation.

Spray drying parameters	Operational conditions
Feed-flow rate	11 mL/min
Inlet temperature (T_{inlet})	160 °C
Outlet temperature (T_{outlet})	81 °C
Drying gas flow rate	38 m^3/h
Spraying gas flow rate	498 L/h

2.2.1. Characterization of spray-dried powders

Particle size distribution was measured by light diffraction using a Mastersizer 2000 equipped with a Scirocco dry disperser (Malvern Instruments, France) at a dispersing pressure of 1 bar. The refractive index used was 1.5. Values presented are the average of at least 3 determinations, and error bars indicate the standard deviation (S.D.). The powder density was evaluated by tap density measurements using a tapping apparatus (Pharma test PT-TD1). Tap density (ρ) was measured in a 10 mL glass graduated cylinder filled with a fixed initial volume of powder around 8 mL. The tap density was determined after 1000 taps from a constant height. Measurements were performed in duplicate. The morphology of particles was examined by scanning electron microscopy (SEM) using a LEO1530 microscope (LEO Electron Microscopy Inc., Thorn-wood, NY) and operating between 1 and 3 kV with a filament current of about 0.5 mA. Powder samples were deposited on a carbon conductive double-sided tape (Euromedex, France). They were coated with a palladium–platinum layer of about 4 nm, using a Cressington sputter-coater 208HR with a rotary planetary-tilt stage, equipped with a MTM-20 thickness controller. The thermal properties of the powders were analyzed using differential scanning calorimetry (DSC) (DSC7, PerkinElmer, USA). Thermograms were analyzed using *Pyris* software. An empty aluminum pan was used as the reference for all measurements. A sample (1–5 mg) of powder was placed in hermetically sealed 40 µL aluminum pan and analyzed. DSC runs were conducted from 30 to 210 °C at a rate of 10 °C/min. Calibration was achieved using Indium (T_{onset} = 156.60 °C) as well as Zinc (T_{onset} = 419.47 °C). The onset and peak temperatures and enthalpy of transition (ΔH) were determined for each peak. Powder crystallinity was analyzed using X-ray powder diffraction (XRPD). XRPD patterns were measured on a Bruker D2 diffractometer equipped with a XFlash detector in SPMS laboratory – Centre of diffraction – École Centrale Paris using Ni-filtered Cu Kα radiation. Data were collected over an angular range comprised between 5° and 40° (2θ) with a step size of 0.01 and a counting time of 5 s/step.

2.3. Powder stability

Powder stability was assessed by leaving samples to age at room temperature under vacuum in a desiccator (temperature comprised between 15 and 25 °C). Particle size was performed on aged samples at t = 0, 2 and 4 weeks.

2.4. Drug content

The content of PZA in the powder was determined by UV–Visible double beam spectrophotometer (Lambda 25, PerkinElmer, France) with 1 cm matched quartz cuvettes. 20 mg powder was accurately weighed and transferred into 20 mL volumetric flask. It was dissolved properly and diluted up to the mark with ethanol/water (70/30). Then, the solution was diluted to obtain a 10 µg/mL solution. The absorbance of the solutions containing PZA was determined in the UV range 200–400 nm using an ethanol/water (70/30) blank. The standard curve was constructed by plotting the absorbance of pyrazinamide from 2 to 16 µg/mL of PZA.

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