International Journal of Pharmaceutics 542 (2018) 72–81

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

The influence of surface active L-leucine and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) in the improvement of aerosolization of pyrazinamide and moxifloxacin co-spray dried powders

Basanth Babu Eedara[a], Bhamini Rangnekar[a], Colin Doyle[b], Alex Cavallaro[c], Shyamal C. Das[a,*]

[a] School of Pharmacy, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
[b] The University of Auckland, 20 Symonds Street, Auckland, New Zealand
[c] Future Industries Institute, University of South Australia, Mawson Lakes, SA, Australia

ARTICLE INFO

Keywords:
Pyrazinamide
Moxifloxacin HCl
L-Leucine
1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine
Surface composition
Aerodynamic performance

ABSTRACT

Pharmacotherapy of tuberculosis is potentially more efficient when delivered by the inhaled route than by the current oral and/or parenteral routes due to the higher concentration of drug reaching the primary region of infection in the lungs. This study investigated the influence of the amino acid L-leucine alone and in combination with the phospholipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), on the aerosolization behaviour of the anti-TB drugs, pyrazinamide and moxifloxacin HCl. Spray dried powders of pyrazinamide (P), moxifloxacin (M) alone and in combination with 10% L-leucine (PL and ML) and 10% DPPC (PLD and MLD) were produced. The particle sizes of all powders except P were in the inhalable size range (< 5 μm) but differ in their morphology in presence of the excipients. X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) revealed the migration of surface active L-leucine and DPPC onto the surface of the particles during the spray drying process. The aerosolization from a dry powder inhaler, Aerolizer®, using a Next Generation Impactor revealed fine particle fraction (FPF) values for P, PL and PLD of 18.7 ± 3.4%, 53.0 ± 3.2% and 74.5 ± 5.3% respectively while FPF values for M, ML and MLD were 55.6 ± 3.3%, 74.7 ± 4.7% and 74.1 ± 1.3% respectively. In conclusion, the differences in the aerosolization behaviours of the pyrazinamide and moxifloxacin spray dried powders with and without excipients was a combination of difference in the surface morphology and surface composition.

1. Introduction

Tuberculosis (TB) remains a major health problem with around 2 billion people currently carrying the causative microorganism, *Mycobacterium tuberculosis* (Mtb). Although treatment for TB has been available for many decades, 10.4 million new cases were reported worldwide and 1.8 million people died of the disease in 2015 (Das et al., 2015; WHO, 2016). The major problem now is the ability of Mtb to develop resistance to anti-TB drugs currently in clinical use giving rise to multi-drug resistant TB (MDR-TB). This necessitates research not only into new anti-TB drugs but also improved methods of delivery of existing drugs.

Since 70–80% of Mtb is localized in the lung, delivery of anti-TB drugs to the lung offers several potential advantages over oral, intramuscular and intravascular routes of administration (Das et al., 2014). In particular, achievement of high drug concentrations in the lung using relatively low doses (Garcia-Contreras et al., 2012) has the potential to increase treatment success, reduce the length of treatment, reduce the risk of drug resistance and reduce systemic toxicity (Gupta et al., 2013; Misra et al., 2011; Ober et al., 2013; Son and McConville, 2011). However, in treating TB, it is important to deliver high doses of drugs to the lung (many milligrams as opposed to < 500 μg for asthma) so that a sufficient quantity reaches the lower respiratory tract where Mtb largely resides. This requires the use of powders which are carrier-free or contain minimum amounts of excipients (high drug-load) and which are made up of particles 1–5 μm in size (Claus et al., 2014) capable of producing an aerosol able to reach the lower respiratory tract. Although easy to produce, such powders suffer from the tendency to form agglomerates due to their high cohesiveness so that strategies are needed to reduce this.

Strategies to reduce agglomeration and improve the aerodynamic performance of 'high drug-load' powders include the use of flow enhancers and coating the particles with a low-cohesive or hydrophobic material (Das and Stewart, 2016). Dry coating of micronized particles

* Corresponding author at: School of Pharmacy, University of Otago, Adams Building, 18 Frederick Street, P.O. Box 56, Dunedin 9054, New Zealand.
E-mail address: Shyamal.das@otago.ac.nz (S.C. Das).

https://doi.org/10.1016/j.ijpharm.2018.03.005
Received 19 December 2017; Received in revised form 6 March 2018; Accepted 6 March 2018
Available online 07 March 2018
0378-5173/

with magnesium stearate is very attractive to achieve high aerosolization (Shi et al., 2015). Another approach to improve aerosolization is the production of particles of the "correct" size in a cost-effective and efficient manner by spray drying through appropriate selection of spray drying conditions and excipients (Claus et al., 2014; Fourie et al., 2008; Tong and Chow, 2006).

The amino acid L-leucine has been widely used as an excipient to improve the aerosolization of spray dried inhalation powders at a concentration as low as 10% w/w (Chang et al., 2014; Rabbani and Seville, 2005; Seville et al., 2007). Being surface active, it adsorbs at the interface of droplets during spray drying and reduce their surface cohesiveness and produce dimpled particles with reduced contact area (Sou et al., 2013; Vehring, 2008; Walton and Mumford, 1999). Only few studies have reported the production of pyrazinamide inhalation powders by spray drying. Kaewjan and Srichana (2016) showed that the inclusion of 20% L-leucine increased the fine particle fraction (FPF) from ~9% to 33%.

Aerosolization of spray dried powders has also been improved through inclusion of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), a long chain saturated, zwitterionic phosphatidylcholine and major component of lung surfactant (LS). In fact, dry powder formulations composed of phospholipids either alone or in combination with other carriers have demonstrated improved aerosolization behaviour (Bosquillon et al., 2001; Cuvelier et al., 2015). Pham et al. (2015) reported such an improvement for pyrazinamide loaded large porous particles composed of hyaluronic acid, L-leucine and DPPC where the FPF was 40.1%. In our laboratory, we have recently produced spray dried powders of the drug using phospholipids (DPPC and DSPE-PEG2K) and L-leucine (Eedara et al., 2016) where inclusion of 25% DPPC increased the FPF from 8.5% to 73.2%. What remains to be discovered is how the combination of L-leucine and DPPC brings about this improvement in aerosolization.

This study aimed to investigate the mechanism by which L-leucine and DPPC influence the aerosolization of 'high drug-load' inhalable powders of two anti-TB drugs, pyrazinamide and moxifloxacin produced by spray drying. Pyrazinamide is a first line anti-TB drug and the only one effective in latent TB (Mitchison and Fourie, 2010). Moxifloxacin is a second line anti-TB drug belonging to the fluoroquinolone class which, if taken concomitantly with a first line anti-TB drug, has the potential to shorten the duration of treatment (Chan et al., 2014). These two drugs were chosen since they differ in their aqueous solubility (pyrazinamide aqueous solubility 50 mg/mL; moxifloxacin HCl aqueous solubility 24 mg/mL) and solid-state nature (pyrazinamide remains crystalline and moxifloxacin transforms to amorphous form upon spray drying). Pyrazinamide, and moxifloxacin were independently spray dried with 10% L-leucine and subsequently with 10% DPPC to achieve high drug loads and evaluated for their morphology, solid-state nature, surface composition and *in vitro* aerosolization behaviour.

2. Materials and methods

2.1. Materials

Materials (suppliers) were as follows: Pyrazinamide (99.7% purity) (Amsal Chem. Pvt. Ltd., Gujarat, India); moxifloxacin HCl (99% purity) (Hubei Yuancheng Saichuang Technology Co. Ltd., Wuhan, China); L-leucine (98% purity) (Hangzhou Dayangchem Co. Ltd., Hangzhou, China); DPPC (1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; ≥99% purity) (Lipoid, Ludwigshafen, Germany); and silicone oil (10 cSt) (Sigma-Aldrich, St. Louis, USA). Hard gelatine/PEG capsules (size 3) were a generous gift from Qualicaps (Osaka, Japan). All other reagents and chemicals were of HPLC grade and purchased from Merck (Darmstadt, Germany). Freshly collected and filtered (0.45 µm membrane filter) Milli-Q water was used throughout the study.

2.2. HPLC analysis

The HPLC system (Shimadzu, Kyoto, Japan) consisted of an SPD-M20A photodiode array detector, LC-20AD solvent delivery unit, DGU-20A5 degasser and SIL-20AC auto-sampler. Classic-VP 7.4SP4 software was used to analyse drug concentration. Chromatographic separation was performed on an octadecylsilyl (ODS; C_{18}) column (150 mm × 4.6 mm, 4 µm; Phenomenex, California, USA) preceded by an ODS security guard column (4.0 mm × 3.0 mm; Phenomenex, California, USA). Both pyrazinamide and moxifloxacin were analysed in the isocratic mode with a flow rate of 1 mL/min and injection volume of 20 µL. The mobile phase for pyrazinamide analysis was 0.1 M sodium dihydrogen phosphate buffer (pH 4.4): acetonitrile (90:10% v/v) and detection wavelength was 269 nm. The mobile phase for moxifloxacin analysis was 30 mM orthophosphoric acid (pH 2.5 adjusted with triethylamine): methanol (55:45% v/v) and detection wavelength of 296 nm. The assays of pyrazinamide and moxifloxacin were linear over the concentration ranges 0.5 to 80 µg/mL ($R^2 > 0.9997$) and 2 to 80 µg/mL ($R^2 > 0.9997$), respectively. Assay validation for both drugs showed good accuracy (± 15%) and precision (< 15%) with limits of detection (LOD) and quantitation (LOQ) of 0.04 and 0.11 µg/mL for pyrazinamide and 0.06 and 0.18 µg/mL for moxifloxacin, respectively.

2.3. Preparation of the spray dried powders

Spray dried powders of pyrazinamide and moxifloxacin (Table 1) were produced using a laboratory scale spray dryer (BUCHI B-290 Mini Spray Dryer, BUCHI Labortechnik AG, Switzerland), filled with a stainless steel standard 2-fluid nozzle with a 0.7 mm (internal diameter) nozzle tip and 1.5 mm cap. The spray dryer was operated in a closed mode using nitrogen gas (British Oxygen Company Gas & Gear, Dunedin, New Zealand). The spray dryer was equipped with a series of the drying chamber, high-performance efficiency cyclone, outlet filter connected to the B-295 Inert Loop via two hose connections. Feed solutions (0.5% w/v; Table 1) were prepared in ethanol-water mixture (70:30% v/v) with bath sonication for 15 min. The feed solutions of the formulations containing L-leucine were prepared by dissolving L-leucine alone in water followed by mixing with ethanol containing other components. The feed solutions were spray dried under the following conditions: Pump flow rate 2 mL/min, air flow rate 670 L/h, aspiration rate 50%, inlet temperature 70 °C; outlet temperature 41 ± 1 °C. All spray dried powders were collected and stored in a desiccator at room temperature when not in use. Process yields (%) of the collected powders are expressed as percentage weight of the powder collected compared to the initial weight of the solids dissolved in the feed solution.

2.4. Drug content estimation

Spray dried powder samples (5 mg) were weighed accurately and dissolved using a mixture of water/methanol (80:20% v/v) in a 100 mL volumetric flask. The flasks were shaken at room temperature for 60 min on an orbital mixer incubator (Ratek Instruments Pty. Ltd., Victoria, Australia) at a speed of 200 rpm followed by bath sonication using an ultrasonic cleaner (Misonix Inc., NY, USA) for 15 min. The

Table 1
Composition of spray-dried powder formulations.

Formulation	%P	%M	%L	%D
P	100	–	–	–
PL	90	–	10	–
PLD	80	–	10	10
M	–	100	–	–
ML	–	90	10	–
MLD	–	80	10	10

P- pyrazinamide; M- moxifloxacin; L- L-leucine; D- DPPC.