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Potential of aerosolized rifampicin lipospheres for modulation of pulmonary pharmacokinetics and bio-distribution

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ABSTRACT

The aim of the present study was to establish the potential of rifampicin loaded phospholipid lipospheres carrier for pulmonary application. Lipospheres were prepared with rifampicin and phospholipid in the ratio of 1:1 using spray drying method. Further, lipospheres were evaluated for flow properties and surface area measurement. The formulated lipospheres were evaluated *in vitro* for aerodynamic characterization and *in vivo* for lung pharmacokinetics and biodistribution studies in Sprague Dawley rats. Powder flow properties finding suggested the free flowing nature of the lipospheres. *In-vitro* aerosol performance study indicated more than 80 ± 5% of the emitted dose (ED) and 77.61 ± 3% fine particles fraction (FPF). Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were found to be 2.72 ± 0.13 μm and 3.28 ± 0.12, respectively. *In-vitro* aerosol performance study revealed the higher deposition at 3, 4 and 5 stages which simulates the trachea-primary bronchus, secondary and terminal bronchus of the human lung, respectively. The drug concentration from nebulized lipospheres in the non-targeted tissues was lesser than from rifampicin-aqueous solution. The pulmonary pharmacokinetic study demonstrated improved bioavailability, longer residence of drug in the lung and targeting factor of 8.03 for lipospheres as compared to rifampicin-aqueous solution. Thus, the results of the study demonstrated the potential of rifampicin lipospheres formulation would be of use as an alternative to existing oral therapy.

1. Introduction

Although tuberculosis (TB) is as old as humanity, it is considered as the foremost cause of death worldwide due to a single microorganism. It is a chronic infectious disease most often due to *Mycobacterium tuberculosis* (MTB) infection (Singh et al., 2014) by inhalation, and the lung is the primary site of infection. Conventional therapy of TB involves prolonged oral administration of high systemic doses of single or combined antibiotics, which often causes unwanted side-effects by high systemic exposure. The administration of antitubercular drugs by inhalation offers an attractive alternative, delivering high concentrations of antibiotic directly to the site of infection while minimizing systemic bioavailability. Rifampicin (RMP) is a lipophilic bactericidal first-line drug used in the treatment of TB. However, poor and unreliable pharmacokinetic profile, severe hepatotoxicity, induced degradation at acidic pH of stomach; patient non-compliance in long-term therapy (Singh et al., 2014) and drug interactions restricts its use. With the idea of increasing the local therapeutic effect and reduce the overall systemic exposure, numerous studies have been proposed for the administration of antitubercular drugs (ATDs) to the primary site of infection, specifically, pulmonary delivery (Ito and Makino, 2004; O'Hara and Hickey, 2000; Sung et al., 2009; Vyas et al., 2004).

Pulmonary delivery *via* inhalation is a common technique of drug administration to patients with a variety of lung diseases (Heijerman et al., 2009; Lavorini et al., 2008; Meenach et al., 2013). This is the favored route of administration of drugs over both

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parenteral and oral route of drug administration. The principal advantages include reduced systemic side effects and higher dose levels of the applicable medication at the site of drug action. Unlike the oral route of drug administration, pulmonary inhalation is not subject to first-pass metabolism. Also, the drugs administered *via* the pulmonary route as inhalation reaches directly to the site of action in the lung such as epithelium and smooth muscles, at high effective local drug concentration resulting rapid onset of action (Labiris and Dolovich, 2003; Patton et al., 2004; Timsina et al., 1994). The efficacy of inhalation drug delivery can be improved by critically formulating drug in the dosage form to meet the physicochemical properties. The crucial parameters that determine the region of deposition in the lung such as particle size, density, shape, charge, hygroscopicity and surface properties, etc. (Heyder, 2004; Timsina et al., 1994). Particles having aerodynamic diameter in the range of 1–5 lm are required for efficient deposition of the particles in the lower regions of respiratory tract (Carvalho et al., 2011; Musante et al., 2002). Particles below 1 μm will exhaled out whereas larger particles deposit in the tracheobronchial (>5 μm) and oropharynx (>10 μm) region of the respiratory tract (Davies et al., 1976; Thompson, 2003). Also, the formulations for pulmonary delivery should be biocompatible and biodegradable during aerosolization with sufficient drug load.

Inclusion of certain surface active excipients in pulmonary formulations may enhance bioavailability, but may also interfere with cell lipid bilayer membranes and thus raise long term safety concerns (Patton et al., 1988). Phospholipids (PLs) are the principle components of all cellular and subcellular membranes. PLs acts as biofunctional surfactants, accounting for their solubilizing property as a carrier system. Also, in the literature it is reported that that water insoluble drugs combined with phospholipids might be beneficial for the improved bioavailability and enhanced biological effect of drugs (Cui et al., 2006; Guo et al., 2014; Maiti et al., 2007; Semalty et al., 2010a,b).

This study was undertaken to evaluate novel and versatile RMP phospholipid lipospheres as dry powder inhalation formulation for deep drug delivery of RMP, which could potential lead to optimization of anti-tubercular treatment. We have evaluated RMP phospholipid complex oral pharmacokinetics and observed enhanced oral bioavailability (Singh et al., 2014). In addition, we have observed the phospholipid complex alleviated RMP-induced hepatotoxicity in animal model (manuscript under review). In this study, we evaluate the potential of RMP lipospheres for aerosolization and deep lung delivery. The advantages of liposphere formulation include: absence of synthetic polymers and surfactants, biocompatibility of phospholipid in the lipospheres, the ease of preparation and scale-up of the liposphere formulation, industrial viability of spray-drying, potential to increase efficacy of treatment by direct drug delivery, reduce RMP dose and toxicity, potential to incorporate other anti-tubercular drugs and reduce duration of treatment with better patient compliance. This technique has the potential for development as a platform technology for lung delivery of drugs for local and systemic action.

2. Materials and methods

2.1. Chemicals

RMP was obtained as a generous gift from Sandoz Pharmaceuticals Ltd., Mumbai. The phospholipids (Lipoid S-75) were gift samples kindly provided by Lipoid Ludwigshafen, Germany. Noscapine was purchased from Sigma–Aldrich, India. Dichloromethane and other chemicals were obtained from Loba Chemie, Mumbai, India. Milli Q water (Nanopure Diamond by Barnstead, Dubuque, IA, USA) was used in all the experiments. HPLC grade methanol was purchased from Merck (Mumbai, India). All other chemicals employed in the study were of analytical grade.

2.2. Formulation design

Dry powder inhalation formulation was prepared by spray drying open cycle system (Buchi Mini Spray Dryer Model B-191, Buchi, Switzerland). The feed solution (0.2% w/v) of equimolar concentration of RMP and phospholipid (PL) in dichloromethane (DCM) was spray dried under optimum conditions. The solution was passed through a stainless steel 0.7 mm diameter atomizing nozzle by a peristaltic pump to obtain 4 mL/min flow rate (pump rate 12%) employing atmospheric gas for drying at a flow rate between 30 and 40 kg/h. The inlet and outlet temperatures were set at $80 \pm 2\,^{\circ}C$ (primary drying step) and $55 \pm 3\,^{\circ}C$ (secondary drying step) with an aspirator rate of 90%. The resultant powder was blown through the high-performance cyclone separator and collected in the sample container. Powder formulation was stored in sealed glass vials at room temperature until further use.

2.3. Physicochemical characterization of dry powder formulation

2.3.1. Bulk and tapped density

A known amount of powder mass was poured into a graduated cylinder, and tapped for defined number of times. For calculation of tap and bulk densities, we noted the initial volume and final volume (after tapping). A 10 mL graduated cylinder was filled with 100 mg of dry powder for testing and tapped until there was no further reduction in volume. The static powder flow was determined by Carr's compressibility index (CI). This was determined by following equation:

$$\mathrm{CI} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \quad (1)$$

2.3.2. Powder flowability

For assessment of powder flowability, measurement of angle of repose is the most frequently adopted method. Powder was poured through a funnel to form cone-shaped pile with an angle, α, to the horizontal. The value of α, was calculated by measuring the height and radius of the pile formed. Powder flowability is inversely proportional to the angle of repose *i.e.*, a large angle of repose is an indicative of poor flow properties while a small angle of repose indicates a free flowing powder. Apart from angle of repose properties, Hausner's ratio was also determined.

$$\mathrm{Tan}\ \alpha = \frac{\text{height}}{0.5 \times \text{base}} \quad (2)$$

$$\mathrm{H.R.} = \frac{\text{tapped density}}{\text{bulk density}} \quad (3)$$

2.3.3. Surface area measurement

To determine the surface area (SMART SORB 91 Surface Area analyzer; Smart Instruments, Mumbai, India), approximately 100 mg of dry powder was dried at 50 °C for 30 min under vacuum and dead volume of sample cell was measured at room temperature. Nitrogen adsorption and desorption measurements were performed using sample cells and empty reference cell immersed in liquid nitrogen. Each measurement was repeated twice to obtain the average surface area calculated by BET equation (Brunauer–Emmett–Teller) method.

$$V_m = V_a\left(\frac{P_0}{P} - 1\right)\left[\frac{1}{C} + C - \frac{1}{C} \times \left(\frac{P}{P_0}\right)\right] \quad (4)$$