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Original Research Paper

Novel potential for optimization of antitubercular therapy: Pulmonary delivery of rifampicin lipospheres

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ABSTRACT

The aim of the present work is to develop rifampicin loaded phospholipid lipospheres containing sulfphobutyl ether β-cyclodextrin and Vitamin C for inhalation to test their potential for deep lung delivery. The findings of the solid state characterization revealed the amorphous nature of the lipospheres. These exhibited a better flowability, an aerodynamic diameter in the range of 1.76 to 3.99 μm. Moreover, the fine particle fraction and emitted dose was found in the range of 68.84–83.73% and 80–93%, respectively. Moreover, lipospheres exhibited enhanced/equivalent efficacy *in vitro* in $H_{37}Rv$ strain. Hence, the results show the potential of lipospheres for pulmonary delivery of rifampicin.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB). According to World Health Organization (WHO), 1.5 million people died from TB, including 360,000 among people who were HIV-positive (WHO Global Tuberculosis Report 2014) [1]. A major paradigm shift in the treatment of TB occurred with the introduction of rifampicin (RMP), the last landmark drug introduced for TB treatment [2]. RMP is the semi-synthetic hydrazine derivative of rifampicin B [3]. In addition to bactericidal effect on MTB, it exhibits excellent late

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sterilizing action on semi-dormant organisms undergoing short bursts of metabolic activity. However, some of the challenges that this potent antibiotic faces include poor water solubility, low oral bioavailability, relatively short biological half-life (1.5–5 h) [3], induction of a prehepatic first-pass metabolism, hepatotoxicity and adverse effects due to multiple doses [4]. Hence, conventional oral treatment of TB is not only long-term [5] but also associated with disadvantages of side effects and systemic toxic effects due to high doses [6]. Vitamin C (Ascorbic acid) (AA) is a water soluble vitamin which is a known antioxidant [7,8] and free radical scavenger [9]. AA has been reported to modulate RMP-induced hepatotoxicity *in vivo* [10] and of other drug molecules as well [11,12]. Moreover, due to the presence of high iron concentration, reactive oxygen species production and DNA damage, AA has sterilizing action on MTB *in vitro* [13]. Cyclodextrins (CDs) are macrocyclic oligosaccharides composed of (α-1, 4)-linked α-L-glucopyranose units [14–16] with a hollow hydrophobic interior and a hydrophilic outer surface. Application of CD in pharmaceutical, food and cosmetic industry is extensive because of its inexpensive and non-toxic nature. In pharmaceutical industry, they have been employed to enhance the drug aqueous solubility and thereby enhance the oral bioavailability of the encapsulated drug [17–21]. However, natural CDs are associated with problems of limited solubility and toxicity related issues. To address this problem, alkyl moieties such as hydroxyalkyl or methyl on free hydroxyl groups of CD were introduced [15,22]. Sulphobutyl ether β-cyclodextrin (SBE-β-CD) is a chemically modified β-CD, cyclic hydrophilic oligosaccharide. Also, the solubility in water for SBE-β-CD is significantly higher than the parent β-CD. Furthermore, SBE-β-CD does not exhibit the nephrotoxicity associated with β-cyclodextrin [8]. Moreover, no cytotoxic effects of SBE-β-CD have been reported [23].

Pulmonary delivery via inhalation is a common technique of drug administration to patients with a variety of lung diseases [24,25]. This is the favoured route of administration of drugs over both 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. Administration of drugs to the lungs via the inhaled route offers rapid onset of action, high local concentration by direct delivery to the airways and hence high therapeutic ratio [26,27]. However, the airway geometry of the lung poses a challenge for delivery into the alveoli. For effective delivery deep into the lung, the particle size should be between 1 and 5 μm [28], with mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of aerosolized particles. Particles above 5 μm are likely to be deposited in the upper respiratory tract while particles below 1 μm could be exhaled during expiration [29].

Therefore, the aim of this study was to evaluate RMP-loaded dry powder formulation containing sulphobutylether β-cyclodextrin (SDRPL-CD), Vitamin C (SDRPL-AA) and combination of both SBECD and AA (SDRPL-Comb). RMP has previously shown interesting potential for treating pulmonary TB infection via inhalation [4,30,31] and was chosen in this report as a model molecule. The reports are not available for inhaled phospholipid based lipospheres of RMP containing sulphobutylether β-cyclodextrin and Vitamin C along with the *in vitro* antimycobacterial efficacy and aerosol performance studies.

2. Materials and methods

2.1. Materials

RMP was obtained as a kind gift by Sandoz, Mumbai. The phospholipid (Lipoid S-75) sample was kindly provided by Lipoid Ludwigshafen, Germany. HPLC grade methanol was purchased from Fisher Scientific, UK. Dichloromethane and other chemicals were obtained from Loba Chemie, Mumbai, India. Vitamin C was purchased from HiMedia, Mumbai. SBECD was obtained as a kind gift sample from Cydex Pharmaceuticals USA. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of spray dry powders

The spray drying open cycle system process was performed using the BUCHI Mini Spray Dryer B-191 attached with high-performance cyclone (BUCHI Labortechnik G, Flawil, Switzerland). Co-spray dried particles (SDRPLs) were obtained by spray drying organic solvent (dichloromethane) and aqueous solutions RMP, PL and SBECD and AA, respectively. The feed solution (3.5% w/w) was passed through a stainless steel 0.7 mm diameter atomizing nozzle via a peristaltic pump at a flow rate of 4 ml/min (pump rate 12%) employing atmospheric gas for drying at a flow rate between 30 and 40 kg/h. A set inlet temperature of 80 °C ± 2 °C (primary drying step) resulted in outlet of 55 °C ± 3 °C (secondary drying step) with an aspirator rate of 90%. The resultant dry powder particles were blown through a high-performance cyclone separator and collected in the sample container. Dry powder formulation was stored in glass vials sealed with parafilm at room temperature.

2.2.2. Fourier transform infra red spectrometry

Fourier transform infra red spectrometry (FTIR) spectra of samples were recorded with a FTIR spectrometer (Nicolet, Impact 410, USA) employed with a denudated triglycine sulphate detector. The spectra were scanned in the region 450–4000 cm^{-1} derived from 11 single average scans. Potassium bromide pellet method was employed for the analysis. The data were collected and analyzed using Omnic 5.1a software (Thermo Nicolet, USA). These are similar conditions to those previously reported [5].

2.2.3. Thermal analysis

Thermograms were obtained on a Mettler Stare System (Mettler Toledo, DSC 821e, Switzerland) using similar conditions previously reported in the literature [5]. Approximately 2–8 mg of powder was carefully weighed into hermetic anodized aluminium DSC pans. An empty, hermetically sealed, anodized aluminium pan was used as reference. Samples were heated at a rate of 20 °C/min over a temperature range of 25 °C to 300 °C. Nitrogen gas at purge rate of 100 ml/min was used as the purging gas.