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Inhalable clarithromycin liposomal dry powders using ultrasonic spray freeze drying

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ABSTRACT

Liposomal dry powder inhalation for the pulmonary administration has a great potential to improve the efficacy of antibiotics while reducing adverse effects. To improve aerosolisation efficiency of liposomal dry powders, we prepared clarithromycin liposomal powder formulations (CLA-Lips-DPIs) by an ultrasonic spray freeze drying (USFD) method using 15% mannitol and 5% sucrose (W:V) as combination lyoprotectants (co-lyoprotectants). The formulation had a porous structure, comprising micron-sized particles with uniform drug content and high drug recovery. Co-lyoprotectants could modulate the liposomal powder from absorbing moisture, resulting in moisture absorption being <15% (W/W) when stored at 75% relative humility for 2 h. The interaction of CLA, lyoprotectant and lipids of CLA-Lips-DPIs was investigated by differential scanning calorimetry. The reconstituted liposome suspension showed a high entrapment efficiency of up to 80% and a narrow size distribution due to the co-lyoprotectants protection. CLA-Lips-DPIs formulations remained unchanged after 3-month storage at 60% RH and 25 °C with a high aerosol efficiency (emitted dose > 85%, fine particle fraction 43%–50%). These results demonstrated the aerosolisation efficiency and storage of the CLA-Lips-DPIs formulation. Liposomal powder formulations prepared by USFD can potentially be an effective drug delivery system for delivering antibiotics.

1. Introduction

Clarythromycin (CLA) is a macrolide antibiotic [1] with a methoxy group (−OCH3) attached to the C6 position of erythromycin.CLA has been widely administrated in the treatment of respiratory tract infections caused bybacteria such as *Pseudomonas aeruginosa*, *Chlamydophila pneumoniae*, *Klebsiella pneumonia*, *Mycoplasma pneumonia*, *Streptococcus pneumonia and Haemophilus in fluenza* [2].CLA has high stability under acidic conditions and low solubility in aqueous solution [3]. The systemic bioavailability of CLA is relatively poor (55%) due to rapid hepatic first pass metabolism [4]. Furthermore, injection of CLA caused clinically significant phlebitis and inflammation in 92% of the participants [5]. It has been shown that the venous irritation of CLA mainly derives from its chemical structure [6]. Administrations of CLA directly to the lungs for the treatment of respiratory infections may minimize these unwanted effects.

Liposomes have been proven to be an effective vehicle for most lipophilic antibiotic drugs due to various potential advantages including universal carrier suitability, sustained release and enhanced intracellular delivery [7–9]. In particular, it is reported that antibiotic-loaded liposomes have superior efficacy against *Pseudomonas aeruginosa* compared with the free drug [10]. Aerosolized liposomal suspension of antibiotic for pulmonary administration will provide the site-specific effects along with prolonged pharmacological response, improved therapeutic efficacy and reduced extra-pulmonary side effects for lung infection treatment. However, liposome suspensions may not be suitable for storage, transportation and manufacturing due to poor stability. Taking the formulation stability into account, liposomal dry powder inhaler formulations (DPIs) stand out for its potential advantages including improved stability, controlled delivery and systemic bioavailability [11–19]. CLA-loaded liposomal DPIs for the pulmonary administration have not been investigated previously.

USFD is one of the methods which can produce large porous particles by using the process of ultrasonic atomization, rapid freezing, and lyophilization [18]. In the USFD process, atomization using an ultrasonic nozzle sprays an aqueous formulation directly into liquid nitrogen, which rapidly freezes the atomized droplets. The frozen particles are collected and lyophilized, leaving behind a dry powder. USFD has the advantages of producing particles with (1) a narrow size distribution and (2) a porous structure after removing water by lyophilization. The USFD technique was previously focused on producing powder formulations for DPIs [20–21]. However, very little has been reported on the production and physicochemical properties such as encapsulation

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efficiency and drug loading [21] of inhalable liposomal dry powders using the USFD method. In addition, sugars such as sucrose, mannitol, lactose, dextrose and maltose, are usually used as lyoprotectants to stabilize liposome particles during the lyophilization process [22–26]. However, most of these sugars being amorphous will add to moisture absorption of liposome dry powders, and seriously impacted the stability of DPIs for the pulmonary administration.

Our present study attempted to apply combination lyoprotectants (co-lyoprotectants) and USFD method to prepare a inhalable clarithromycin liposomal powder formulation-CLA-Lips-DPIs. Co-lyoprotectants containing mannitol and sucrose were added to study their protective effects on (1) lyophilization of the liposome suspension, and (2) moisture sorption of the lyophilized liposome powders. The stability of CLA-Lips-DPIs was assessed after 3 months of storage at 25 °C and 60% relative humidity. Furthermore, reconstituted liposome suspensions were characterized with respect to the liposome morphology, drug entrapment efficiency (EE) and particle size distribution were evaluated in vitro.

2. Materials and methods

2.1. Materials

Clarithromycin, with over 99%purity, was purchased from Ouhe Chemical Technology Co., Ltd. (Beijing, China). Sucrose (Suc), trehalose (Tre) and mannitol (Man) were obtained from Sigma-Aldrich-Fluka (Buchs, Switzerland). Soybean phosphatidylcholine (SPC) and cholesterol (Chol) were supplied from Tywei Pharmaceutical Co., Ltd. All other reagents were of analytical grade.

2.2. Preparation of CLA-Lips

CLA-loaded liposomes (CLA-Lips) were prepared by thin lipid film hydration and sonication method. Briefly, a mixture of SPC/Chol/CLA (4/1/2, by mass) was dissolved in chloroform and dried into a thin film by a rotary evaporator (R-210, BÜCHI Labortechnik AG, Flawil, Switzerland). The film was desiccated under vacuum overnight, and hydrated with distilled water at a 50 °C water bath. The resulting solution was sonicated at 400 W for 5 min, and then filtered through a 0.8 μm filter membrane.

2.3. Characterization of CLA-Lips suspension

The liposome suspension was negatively stained with 3% Ammonium Molybdate and the morphology of the particles was examined by transmission electron microscopy (TEM) (JEM-1200EX; Japanese Electric, Tokyo, Japan). The average diameter and polydispersity index of the liposomes suspension were determined by light scattering technique (Zetasizer2000, Malvern Instruments Ltd., Worcestershire, Britain). The EE of CLA loaded liposomes was assayed by mini-column centrifugation method [27]. The unloaded CLA was removed by gel filtration over a Sephadex G-25 mini-column (2.5 × 0.8 cm).

2.4. Production of CLA-Lips-DPIs

USFD was used to produce the liposomal dry powder [28]. A digitally controlled syringe pump (Model PHD 2000, Harvard Apparatus, Holliston, MA) was used to drive the liposomal formulation liquid into an CV-24 ultrasonic spray nozzle which was powered by a Vibra Cell 40 kHz ultrasonic generator(Sono-Tek Corp., Milton, NY) to atomize the liquid into fine droplets. CLA loaded liposomal suspension containing different kinds and amounts of lyoprotectants (Suc, Man and Tre) was supplied to the nozzle at a constant flow rate of 1 mL/min, and the liquid was atomized with high-frequency sound waves (48 kHz) into liquid nitrogen. The outlet of the nozzle was positioned at about 10 cm above the liquid nitrogen. The frozen particles in liquid nitrogen were transferred into a Christ Alpha 1–4 LOC-1 M freeze dryer (Martin Christ, Osterode am Harz, Germany). The whole process and apparatus of USFD was shown in Fig. 1. The product was dried at 0.04 MPa of the vacuum for 48 h. After removing the samples from the freeze drier, the resulting powder was collected in a humidity chamber with RH ≤ 20% and stored over silica gel in a vacuum desiccator at room temperature. For storage stability, the CLA-Lips-DPIs were subpacked in individual vials and stored at 20–25 °C and the specified RH. For characterization of the reconstituted CLA-Lips-DPIs in liquid, the formulations were reconstituted in pH 6.5 PBS to form the liposome suspension.

2.5. Morphology characterization

Scanning electron microscopy (SEM) was employed to investigate the morphology of the spray-dried formulation powders. Each

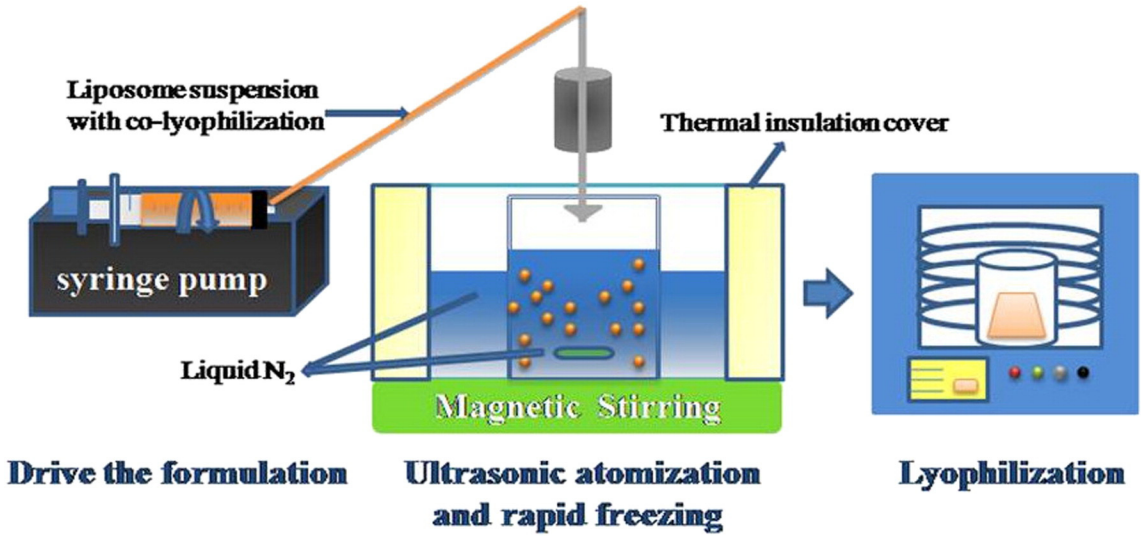

Fig. 1. Schematic diagram of the apparatus and preparation process of ultrasonic spray freeze-drying.