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Cationic, amphiphilic dextran nanomicellar clusters as an excipient for dry powder inhaler formulation

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ABSTRACT

Effective delivery of drugs to alveoli in a controlled manner using hydrophobic polymers as carriers has already been reported. Preclinical studies revealed that toxicity and hydrophobicity are related to each other in pulmonary delivery. Here, we are reporting a chemically modified dextran having amphiphilicity and cationicity achieved by controlled grafting of stearyl amine. Two proportions of lipopolymers were synthesized and physico-chemical characterization was carried out. *In vivo* evaluation of sub-acute toxicity of the synthesized lipopolymer in Sprague–Dawley rats was carried out for three months. This was followed by a histological evaluation of the sacrificed animal's lung. Further, the synthesized lipopolymer was formulated with drug (Rifampicin) loaded inhalable microparticles through spray drying. The final drug formulation was tested for toxicity and proinflammatory responses in human cell lines. Dose deposition efficiency of the formulation was determined using Anderson Cascade Impactor.

1. Introduction

The potential of lung as a port for drug delivery was recognized in the ancient era itself [1]. The large surface area along with extremely thin and delicate physiological barrier between air and systemic circulation (in alveoli) increases the bioavailability of the administered drugs [2]. Inhalational drug delivery can be a method of choice for ailments affecting the lung due to its increased drug availability with minimal systemic side effects. Recently, dry powder inhalation (DPI) formulation gained greater attention over other inhalational formulations as it has increased patient compliance, formulation stability and environment friendliness. The biggest challenge in targeting alveoli is to overcome the effective filtering mechanism of the respiratory system. It is reported that particles of size range 1–5 microns succeed in reaching alveoli, whereas particles of ultrafine size (less than 1 micron) fail to settle in the alveoli and get exhaled out [3]. The most widely accepted strategy in DPI technology is admixing the micronized active pharmaceutical ingredient (API) with lactose [4]. Emitted dose variability and low efficiency in dose delivery (approx. 20% of label claim) are major limitations of this strategy [5,6]. Dose variability is not a serious problem for the symptomatic treatment of asthma as most of these currently available formulations are meant to target the upper respiratory tract.

Hydrophobic microparticles made of poly (D,L-lactide), polycaprolactam, poly (lactide-co-glycolide) are proven efficient carriers for DPI [7–9]. These systems are found to have controlled release of drugs in alveoli. But, these particles are prone to macrophage clearance from the alveoli. Nanoparticles can escape from macrophage and mucociliary clearance [10]. In addition to this, nanoparticles have a higher surface area to mass ratio when compared to microparticles. This is very desirable for wider distribution of the drug in the lung. Edward group reported a two step method, in which nanoparticles were prepared and were further formulated to hollow microspheres of inhalable range [11,12]. Nanonization often imparts an enormous increase in Gibb's free energy due to the increase in surface area [13,14]. This leads to poor redispersibility of the nano clusters. To overcome this, in another embodiment, a cryo-protectant was added to the nanoparticle suspension and was further spray freeze dried [8]. As a drug carrier silica nanoparticles were spray dried with water soluble excipients to form microparticles. They demonstrated the redispersibility of the microparticles into individual nano carriers in aqueous environment [15].

Micelles constitute a thermodynamically stable system and attain its morphology due to the balance between hydrophobicity and hydrophilicity. Clinical trials of Genexol PM have demonstrated that amphiphilic biodegradable and biocompatible

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polymeric micelles have better efficiency in delivering anti-cancer drugs to tumors [16]. Copolymerization of hydrophobic and hydrophilic polymers or grafting of hydrophobic moieties on a hydrophilic polymeric backbone is the widely used method for polymer micelle preparation [17–20]. It is possible to achieve the size of micelles in nano range by tuning the hydrophilic and lipophilic balance [21]. Biocompatible polymers such as pluronics and caprolactone are reported to be effective in delivering drugs to the lung [8,22]. Carbohydrate polymers such as chitosan and dextran are better carriers in biomedical applications due to their biodegradability and biocompatibility. Primary amino functionalities in chitosan confer insolubility in neutral pH. Because of this, chemical modification of the chitosan polymer is possible only under mild acidic environment [23]. Dextran is another carbohydrate polymer which is clinically used for systemic administration as a plasma expander. Du et al. has reported that micelles of stearic acid grafted dextran are good carriers for cancer chemotherapy [21].

In clinical practice, formulations intended for systemic use can be administered to the lung (off-label use) through nebulization [24]. In accordance with this, after preclinical studies Bend research Inc. proposed dextran as a suitable DPI excipient [25]. Osman et al. made use of chitosan to impart mucoadhesive properties to inhaled dextran sulfate microparticles [26]. Hydrophilic polymers are however recognized as poor drug carriers due to their burst release properties. Microparticles made of chemically modified, hydrophobic acetylated dextran are observed to have sustained release of the entrapped drug on *in vitro* release kinetics [27].

Here, we are reporting synthesis of a cationic amphiphilically modified dextran through grafting hydrophobic stearyl amine in a two step reaction. To study the effect of grafting on physico-chemical as well as safety aspects, two different combinations were synthesized by changing the mass ratio of stearyl amine to dextran. This lipopolymer was further formulated to drug containing inhalable microparticles through spray drying. In the present work, we have developed a system having the advantages of both micro and nanoparticulate inhalational systems. The optimum aerodynamic size of spray dried, drug loaded particles will help in the effective alveolar deposition. Further, the presence of lung fluid will steer the deposited microparticles to individual stable nanomicelles due to amphiphilic and cationic properties of stearyl amine grafted dextran. This acts like a micron sized cluster bomb which releases the nano drug carrier after reaching the target site.

There are many reports emphasizing the need for inhalation formulation for treating tuberculosis (TB) [28]. The widely accepted mode of TB treatment is prolonged intake of fixed dose combination (FDC) tablet containing rifampicin, isoniazid, ethambutol and pyrazinamide. Among the first line of these anti-TB drugs, rifampicin is the only hydrophobic one [29]. This causes dissolution rate limited bioavailability of rifampicin (BCS class II drug). Apart from this, rifampicin often shows chemical and physical incompatibility with the ingredients of the FDC tablet. Moreover, avascularity and remodeling of lung in TB patients aggravate the situation through defective drug delivery to the target site [30]. All this made us choose rifampicin as a model drug for the present newly developed DPI formulation.

2. Materials and methods

2.1. Materials

Dextran of average molecular weight 40,000 Dalton, Zinc tetrafluoroborate, Roswell Park Memorial Institute (RPMI) 1640 media, Rhodamine 123, Phorbol myristate acetate (PMA), Eosin, Hematoxylin and Phosphate buffered saline (PBS) were purchased from Sigma (USA). Epichlorohydrin was purchased from Spectrochem (India). Stearyl amine or Octadecyl amine was procured from SRL (India). All solvents used were purchased from Merck (India). SERVAPOR® dialysis tubing (21 mm diameter) with a molecular weight cut-off of 12,000–14,000 Daltons was obtained from SERVA Electrophoresis (Germany). Inhalable grade lactose (Inhalac 230) was provided by Meggle Pharma (Germany) as a gift sample. Rifampicin (RIF) and Müller Hinton broth were purchased from Hi-media Laboratories (India).

2.2. Synthesis of stearyl amine grafted dextran

Clinically used dextran of molecular weight 40,000 Daltons was used for the synthesis. The lipopolymer was synthesized through a two step reaction. Initially, the dextran was functionalized to chlorinated dextran using an early reported method [31]. In brief, dextran was dissolved in minimum quantity of water. This was reacted to epichlorohydrin in the presence of an acid catalyst (Zinc tetrafluoroborate 25% aqueous solution). The reaction was carried out at 90 °C for 3 h and further the polymer was purified through dialysis against Milli Q® water using a dialysis tube. After 72 h of dialysis, the chlorinated dextran was lyophilized to a fluffy white powder. The extent of binding of stearyl amine on to dextran is directly proportional to the number of available chlorine atoms. In preliminary experiments we observed that an increase in the extent of stearyl amine grafting hampers the hydration of bulk polymer and also prevents the lipopolymer from micelle formation. So, for forming more stearyl amine grafted dextran micelle (Dex-HS), 140 ml of epichlorohydrin was reacted with 10 g of dextran. To study the effect of lower extent of grafting (Dex-LS), 70 ml epichlorohydrin was used to functionalize 10 g of dextran.

Thermal amination method was adopted for grafting stearyl amine on to chlorinated dextran [32]. Chlorinated dextran, along with stearyl amine was dissolved in a common solvent i.e. dimethylsulfoxide (DMSO). The reaction was carried out at 90 °C for 3 h. Unreacted stearyl amine was extracted to chloroform using a separating funnel. For Dex-HS the quantity of stearyl amine used was 3 g whereas it was 1.5 g for Dex-LS (Scheme 1).

2.3. Fluorescent tagged stearyl amine grafted dextran for in vivo biodistribution study

To determine the bio-distribution of particles inside the lung, rhodamine conjugated stearyl amine grafted dextran was synthesized for fluorescent imaging of lung tissue sections. During the thermal amination process, rhodamine 123 was added in trace quantity to obtain a fluorescent tagged lipopolymer. Extensive dialysis (till the dialysate becomes almost non fluorescent) was performed to remove all the untagged rhodamine from the product. This is very crucial to avoid background noise in fluorescent images imparted by free rhodamine.

2.4. Physico-chemical characterization of Dex-HS and Dex-LS (Bulk lipopolymer)

The formations of lipopolymer combinations were confirmed through ^{1}H NMR spectroscopy (Bruker 500 MHz, Germany) using DMSO as solvent. To confirm the hydroxyl groups D_2O exchange was also carried out.

Differential Scanning Calorimetry (DSC 6000, PerkinElmer, USA) was employed for determining the nature of the synthesized lipopolymer. Accurately weighed sample was sealed in an aluminum pan and was scanned while heating between 0 and 250 °C at a rising rate of 10 °C/min. Nitrogen at 20 ml/min was purged into the system. Cooling cycle was also recorded at

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