Powder Technology 291 (2016) 366–374

Contents lists available at ScienceDirect

Powder Technology

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

Pulmonary delivery of rifampicin microspheres using lower generation polyamidoamine dendrimers as a carrier

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ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form 23 December 2015
Accepted 24 December 2015
Available online 6 January 2016

Keywords:
Polyamidoamine dendrimers
PAMAM
Rifampicin
Dry powder inhaler (DPI)
Aerodynamic behavior
Pulmonary absorption

ABSTRACT

Different generations (G1, G2 and G3) of polyamidoamine dendrimers (PAMAM) were synthesized and co-spray dried with rifampicin to produce inhalable microspheric particles for pulmonary delivery. The particle size distribution, morphology and density of the designed formulations were characterized by laser diffraction, scanning electron microscopy (SEM) and helium densitometer, respectively. The aerosolization performance of these formulations was investigated using an Andersen cascade impactor. The formulations were efficient aerosols having favorable fine particle fraction (FPF) and emitted fraction (EF), suggesting that the powders were suitable for inhalation. The absorptions of rifampicin following pulmonary administration of different formulations were also examined using an *in situ* pulmonary absorption method. The pharmacokinetic profiles of different rifampin formulations were studied following intrapulmonary administrations for 60 h. The pharmacokinetics parameters such as C_{max}, t_{max}, $t_{1/2}$, mean residence time (MRT) and the *AUC* were calculated separately. It was evident that the t_{max} value of the formulations was decreased while C_{max} value was increased followed by PAMAM dendritic generations increased from G1 to G3. The lower generation PAMAM microspheres were found to have significant impact on the pharmacokinetics parameters of rifampicin and ultimately drug bioavailability. In this study, PAMAM G3 dendritic microsphere was identified as suitable drug carriers for the pulmonary delivery of rifampicin into lung tissues.

1. Introduction

Pulmonary route is widely being employed to deliver drug molecules into the airways for the treatment of local disorders such as asthma, bronchitis, chronic obstructive pulmonary disorders (COPD) and respiratory infections. There have also been many attempts and increasing interest for systemic delivery of macromolecules through pulmonary delivery [1–4]. To achieve the desired therapeutic effect, drug particles have to travel, deposit and penetrate into the appropriate part of the lungs depending on their particle size, size distribution of carriers containing drug, inhaler device and formulation techniques. Spray drying, is one of the most suitable techniques once the specialized particle type is required and becomes feasible alternative to conventional methods which are already used. Moreover, being a continuous production method, it can be more emphasized upon for large scale production of drug products [2–4].

Tuberculosis (TB), a chronic respiratory infectious disease caused by *Mycobacterium tuberculosis* (Mtb), stands among the world's top causes of mortality [5]. Poor patient compliance is a common obstacle associated with TB treatment [6], which is a lengthy procedure with severe adverse effects including hepatic microsomal autoinduction of oxidative enzymes, gastrointestinal tract (GIT) related side effects, intestinal bacterial flora imbalance, hepatic toxicity in chronic treatment, *etc.* [7–8]. To withstand the aforementioned issues, different therapeutic approaches have been evaluated during the last decades [9–12].

Abbreviations: PAMAM, polyamidoamine; SEM, scanning electron microscopy; FPF, fine particle fraction; EF, emitted fraction; MRT, mean residence time; COPD, chronic obstructive pulmonary disorder; TB, tuberculosis; Mtb, *Mycobacterium tuberculosis*; GIT, gastrointestinal tract; DPIs, dry powder inhalers; XRD, X-ray diffraction; FPD, fine particle dose; ED, emitted dose; MIC, minimal inhibitory concentration.

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http://dx.doi.org/10.1016/j.powtec.2015.12.037
0032-5910/

Rifampicin is a first line drug for the treatment of TB. However its conventional oral dosage forms have serious limitations, such as poor and variable bioavailability [13], low water solubility [14], drug instability in the gastrointestinal fluid [15] and high dosage requirement. Pulmonary delivery of the anti-TB agent could potentially provide an alternative to overcome these problems.

The use of microparticle-based drug delivery systems to achieve specific targeting to the site of TB infection in order to reduce systemic toxicity has been extensively investigated. These formulations can target alveolar macrophages, where Mtb commonly resides [16–18].

To exert its therapeutic effect, rifampicin has to enter the cell and abides adjunct to the Mtb that has been phagocytized and trapped in its live state inside the host cell. As far as the treatment of pulmonary TB concerns, direct delivery of the drug molecules to the site of infection using inhaled aerosols is a highly applicable approach. It could bypass the first pass metabolism and maintain local therapeutic effective concentration followed by decreasing the systemic side effects [19,20].

The use of dry powder inhalers (DPIs) is a popular method to achieve drug delivery to the lung [21–22]. Micronization is a technique frequently used to reduce the particle size of drug powders, bringing them to less than 5 μm, which is required for efficient lung deposition. However powders of this size range exhibit hefty interparticulate cohesion, leading to poor powder flow properties [23,24]. Furthermore, factors that are known to influence the aerosolization properties of dry powders, *e.g.* particle morphology, densities and surface composition [25], are arduous to be controlled effectively during this micronization process. Researchers have investigated a number of approaches to improve powder aerosolization, such as mixing the micronized drugs with other inert carriers [26–28], modification of particle surface morphology [29–30], particle surface roughness [31], particle porosity alteration [32] and powder density [33]. Spray drying technique has also been investigated by many researchers to produce dry powders for inhalation [34–35]. It offers potentials to incorporate a wide range of excipients into the formulation to be co-spray dried, such as dispersability enhancers (*e.g.* leucine [36]) or drug release modifiers (*e.g.* hydroxypropyl cellulose [37], glyceryl behenate [38] and poly(lactic acid) [39]).

Polyamidoamine (PAMAM) dendrimers — introduced by Tomalia [40] — exhibit outstanding properties including high degree of uniformity, narrow Mw distribution, specific size and characteristic shape [41], which make them potential carriers for a wide variety of therapeutic agents [42].

PAMAM dendrimers are not affected by environmental conditions such as temperatures, pH and ionic strength. Moreover, the possibility of aggregation is reduced. Incorporation of rifampicin into PAMAM microspheres not only could retard the drug release rate, but also prolongs the drug residence time in the lung tissues [43]. Rifampicin as a zwitterion molecule exhibiting two pKa 1.7 (4-hydroxyl group) and 7.9 (4-piperazine nitrogen) in water. Lower generation polycationic amine terminated PAMAM, *i.e.* below generation 4 (<G4), have shown to be more biocompatible than higher generations [44–46]. Despite superior encapsulation efficiency of higher PAMAM generations (G≥4) owing to large amount of empty spaces present inside the molecules, the lower generations of PAMAM could modify drug release profile more easily *via* electrostatic interaction between surface groups of the polymers and the ionic drugs. Moreover, although the cationic nature of the dendrimers could improve the bioavailability absorptive characteristics of drug loaded microparticles, non-PEGylated carriers may lead to some extent of cytotoxicity. But the in case of PAMAM dendrimers, encapsulating the drug molecules in the void space of the structure, owes to the reduction of drug toxicity and facilitates the controlled release [47–48].

In this study, generation one (G1), two (G2) and three (G3) PAMAM dendrimers were synthesized and subsequently used as carriers and drug release modifiers for pulmonary delivery. Their effects on the aerodynamic properties of rifampicin [49–50], after co-spray drying, were also examined followed by *in vitro* and *in vivo* studies.

2. Materials and methods

2.1. Materials

Rifampicin was supplied by Sigma (USA) and Lactose monohydrate as the filler of a capsule was purchased from DMV (NE). All other solvents and monomers used throughout the study were supplied by Merck (DE) and Sigma Aldrich (DE) and were at least analytical grade.

2.2. Methods

2.2.1. Synthesis of lower generation polyaminoamine (PAMAM) dendrimers

Tomalia's *in situ* divergent method was followed for the synthesis of PAMAM Starburst dendrimers of various full and half generations, ranging from G = 0.5 to G = 3 [40]. PAMAM dendrimers were synthesized from core reagent in two different steps. To produce lower generations of PAMAM dendrimers, aliphatic or aromatic ester initiator cores including methyl ester of p-methoxy benzoic acid as well as methyl laurate were used. Ethylenediamine was used for amidation step due to its high boiling point. Azeotropic and wiped film distillation techniques were further used to remove the trace amounts of the remained ethylenediamine particularly if higher dendritic PAMAM generation's synthesis was concerned [40].

2.2.2. Preparation of PAMAM G1–G3 dendritic microspheres containing rifampicin by spray drying

The aqueous solution containing rifampicin and each of the polycationic dendrimers, *i.e.* PAMAM G1, G2 or G3, was prepared by dissolving rifampicin 0.25% w/v and polyamidoamines 0.5% w/v in distilled water. The solution was then mixed for 30 min at 25 °C and sonicated for 20 min. The solution was then spray dried, using a lab scale spray drier (Buchi 191, Buchi, CH) with an inlet temperature of 100 °C ± 2 °C, outlet temperature of 90 °C ± 2 °C, air flow rate at 800 Nl/h and pump setting 5 mL/min. The perused feed rate was 5 mL/min and the total volume of the liquid was 50 mL.

2.2.3. Filling of rifampicin microspheres blend into the inhalable capsules

To overcome the likely adhesiveness of PAMAM-rifampicin microspheres as well as prevention of size growth during storage and before filling them into the inhalable capsules, lactose monohydrate was used. The ratio of lactose:microspheres in the final blend was 1:2. The powder blend was then mixed for 10 min in a double cone blender. Further studies were performed as stated in Section 2.3.2.1 of the same paper to ensure the uniformity of the content.

2.3. In vitro characterization of the rifampicin loaded microspheres

2.3.1. Morphological studies

2.3.1.1. Particle size distribution analysis. The particle size distributions of the spray dried powders were determined using a laser diffraction apparatus (Mastersizer X, Malvern, UK). Approximately 20 mg of each sample was suspended in cyclohexane:ethanol in ratio of 80:20 and was then sonicated for about 4 min. It was noted that rifampacin was not soluble in the suspension medium. After 100× dilutions, approximately 1 mL of the samples was analyzed at 25 °C in triplicate.

2.3.1.2. Scanning Electron Microscopy (SEM). The morphology of the spray dried powders was examined by Scanning Electron Microscopy (SEM) (Philips XL 30 scanning microscope, NE) at 20.0 kV. Samples were directly placed onto an aluminum stub using double sided adhesive carbon disc (SCD005 Sputter coater, Bal-Tec, DE). The samples were sputter coated with gold 20 mA for 4 min prior to analysis.