Development and Characterization of Nanocrystal-embedded Microparticles for Pulmonary Delivery of Budesonide

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Summary

Nanoparticle technology represents an effective approach for formulating poorly water-soluble pulmonary medicines. But unfortunately, directly using nanoparticles for inhalation often suffered from the problem of physical instability if they are applied in liquid form, or nanoparticles are likely to be exhaled before deposition if applied in the form of dry powder. In addition, rapid dissolution of nanoparticles will cause the rapid absorption of medicine, which means the rapid clearance of medicine from local lung tissue. To address these problems, a dry powder inhalation formulation composed of nanocrystal-embedded microparticles with hyaluronic acid as matrix excipients was designed for the pulmonary delivery of budesonide. Nanosuspension of budesonide was prepared by using wet milling method and by varying the rotation rate of milling bowl and milling time, three types of budesonide nanosupension were obtained. The nanosuspension was further spray dried with hyaluronic acid into so-called budesonide nanocrystal-embedded microparticles. The crystalline state of budesonide in nanosuspension or microparticles was confirmed by using XRPD and DSC. The morphology of microparticles was observed with SEM and it was shown that the microparticles were round in shape and appeared as wrinkled or crumpled spheres, with some degree of eccentricity. No significant difference between the formulations with different size of nanocrystals was observed. In vitro inhalation performance of microparticles was examined using the NGI system and demonstrated that the obtained nanocrystal-embedded microparticles has good aerosolization properties and suitable size for inhalation.

Introduction

Budesonide (BUD), a potent corticosteroid with high topical anti-inflammatory activity, is one of the mainly used corticosteroids to treat asthma and COPD. Pulmonary delivery is a preferred way for the treatment of these lung-restricted diseases since it could provide relatively high local drug concentration and low systemic exposure. Both of MDI and dry powder inhalation formulations of BUD are currently available in the market. However, hydrophobic nature of the drug is an important obstacle in its clinical use. In the case of dry powder inhalation, the low solubility of BUD would cause low drug concentration in lung and subsequently compromise the therapeutic performance of BUD. The strategy of narrowing the size of particle down to nano-scale was promising in improve the dissolution of BUD[1], and the nebulized BUD nanosuspension was shown to be superior to the micronized dosage form in terms of droplet aerodynamics, cellular uptake and efficacy [2]. But unfortunately nanocrystal used in nebulizers often encounters the problem of physical instability in the form of uncontrolled agglomeration or Ostwald ripening. Processing such suspensions into dry powders could achieve better stability but it can also yield undesirable broad particle size distributions and large portion of powder could be exhaled due to the too small particles size[3]. In addition, for the poorly soluble drug BUD, although rapid dissolution would mean higher local drug level in lung, the maintenance of high local drug level would not last for a long time period due to the high permeability of BUD. BUD is a biopharmaceutical classification system class II drug, and the dissolved drug will be absorbed into systemic circulation rapidly and exert unwanted side effects.

To resolve these problems, a system of nanocrystal-embedded microparticles with hyaluronic acid (HA) as matrix materials was designed. HA, a naturally occurring polymer has excellent bioadhesion by anchoring to mucous constituents, which can delay mucociliary clearance in the lung. HA with high molecular weight can form hydrogel in water and provide sustained release profiles for entrapped drug[4]. We hypothesised that by incorporating BUD nanocrystal into HA microparticles, the release of BUD could be controlled and the local resident time of BUD is expected to be prolonged. The aim of current study is to obtain an inhalable HA dry powder formulation with BUD nanocrystals embedded. Nanocrystals-embedded microparticles were prepared and characterized, and the *in-vitro* inhalation performance of microparticles was also examined.

Experimental methods

1. Materials

Budesonide was purchased from Hubei Gedian Humanwell Pharmaceutical Co.Ltd. China. Pluronic F-68 was a gift from BASF, China. Hyaluronic acid (Mw~1,470,000 Da) was purchased from Shandong Freda Biochem. Co. Ltd. (Jinan, China). The other reagents and solvents used were of analytical grade.

2. Preparation and Characterization of BUD nanosuspension

The budesonide nanosuspensions were prepared by a wet milling technique. Briefly, 0.3 g F68 was dissolved in 30 ml of water. Then, 1 g of budesonide was dispersed in the stabilizer solution. The drug suspensions were pipetted into the milling bowl containing 100 g of milling pearls (zirconium oxide, 0.5mm in diameter). Two milling bowls were fixed in a planetary milling machine (Pulverisette 7 Premium, Fritsch Co., Germany). The grinding was performed at various rotation rates for different time to get BUD nanosuspension with different size. The BUD nanoparticles was collected by centrifugation (Thermo fisher) at 16000rpm for 15min and re-dispersed into distilled water to removed F-68 and be ready for size measurement and following processing.

The mean particle diameter (Z-average) and polydispersity index (PDI) were determined by photon correlation spectroscopy (PCS, Malvern Nano ZS Malvern Instrument, UK). The crystalline form of BUD nanoparticles was analysed using X-ray powder diffraction (XRPD) analysis and differential scanning calorimetry (DSC).

3.Spray drying of nanocrystals into nanoembedded microparticles

Spray drying was applied to incorporate the nanocrystals into nanoembedded using HA as excipient. A total amount of 100 mg BUD nanocrystal were dispersed in 100 ml water with 350 mg HA and spray-dried with a co-current Büchi B-290 spray-dryer (Büchi Labortechnik, Flawil, Switzerland). The process parameters of spray drying were listed in table1.

Table 1.The process conditions of B-290 spray dryer

Inlet temperature (°C)	Aspiration rate (%)	Feed rate (%)	Q-flow (mm)	Feed volume (mL)
140	100	15	40	200

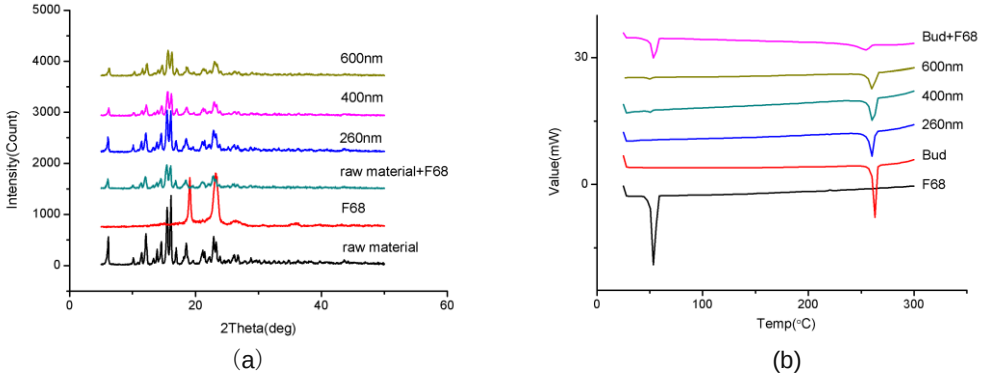
4. Characterization of nanocrystal-embedded microparticles

The Morphology of the obtained dry powder of nanocrystal-embedded microparticles was observed with SEM and the physical state of BUD in microparticles was analyzed with XRPD and DSC. Aerodynamic particle size measurements were carried out according to the European Pharmacopoeia (Apparatus E) using a Next Generation Pharmaceutical Impactor (NGI) (Copley Scientific, Nottingham, UK). 10mg of particles were dispersed using an Cyclohaler® DPI (Pharmachemie B.V., Netherlands) at a constant air flow rate of 100L /min

Results

1. BUD nanocrystals with different size were obtained

In order to examine the size effects of nanocrystal on the characteristics of final spray dried microparticles, three kinds of BUD nanocrystals with various diameters (around 260, 400, and 600nm respectively) were obtained by keeping the formulation constant but varying the rate of rotation and milling time (shown in table 2). The size distribution indicated by polydispersity index (PDI) was narrow for all of the three formulations. According to the X-ray diffraction patterns, as shown in Fig. 1a, it was confirmed that no substantial crystalline change was found in the nanocrystals compared with raw crystals. However, the differences in the relative intensities of their peaks might be attributed to differences in the degree of crystallinity of the samples. DSC was also performed to analyze the different samples (Fig. 1b). In all cases, the DSC scanning of each sample showed a single sharp endothermic peak ascribed to the melting of the drug, which also indicated that there was no substantial crystalline change.



(a) (b)

Fig. 1 XRPD (a) and DSC (b) curves of budesonide crystals with different particle sizes, raw BUD materials, and physical mixture of raw BUD materials and F68

Table2. The size and size distribution of BUD nanocrystals prepared under different process conditions (n=3, Mean±SD)

Formulation No.	Speed (rpm)	Time (min)	Particle size (nm)	PDI
1	200	6	606.77±7.03	0.202±0.067
2	200	31	406.30±24.98	0.127±0.054
3	500	120	267.97±6.07	0.268±0.026

2. Characterization of nanocrystals—embedded micropartlces

The dry powder manufactured by spray drying has good flowability and appeared as wrinkled or crumpled spheres, with some degree of eccentricity (shown in Fig 2). It seems like the size of embedded nanocrystal has no significant influence on the morphology of microparticles. Again, the results of XRPD and DSC (Fig. 3) demonstrated that no substantial crystalline change happened during the process of spray drying.

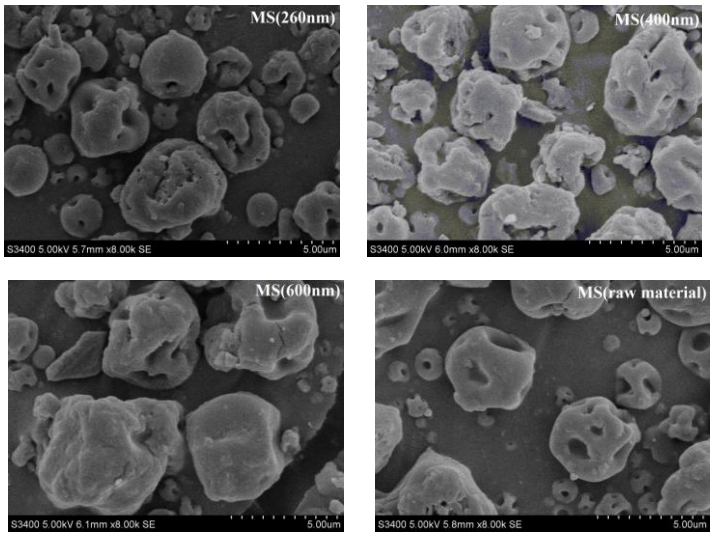
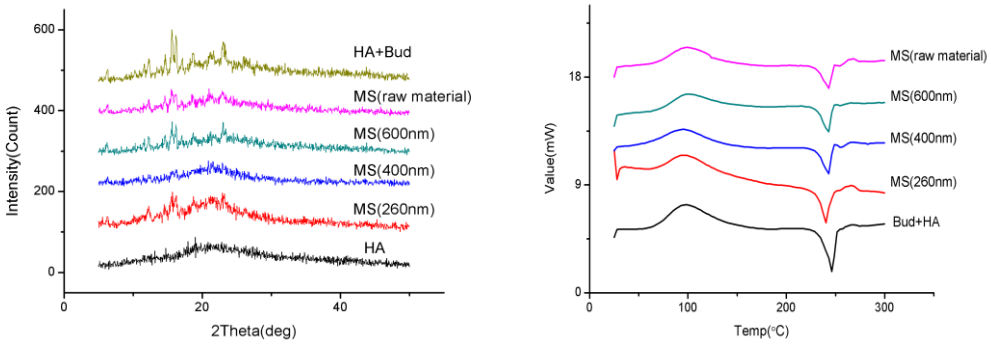


Fig. 2 SEM image of microparticles containing BUD nanocrystals with different size and raw BUD material



(a) (b)

Fig.3 XRPD (a) and DSC (b) curves of HA microspheres containing budesonide crystals with different particle sizes, raw BUD materials, HA alone and physical mixture of raw BUD materials and HA

Table.3. Aerodynamic performance of microparticles containing three kinds of nanocrystals or raw material

No.	MMAD(mm)	GSD(mm)	FPF%
MS(260nm)	5.32	1.68	37.47
MS(400nm)	5.35	1.64	30.19
MS(600nm)	5.23	1.66	31.53
MS(rm)	3.12	6.09	38.97

For further characterization, the in vitro inhalation performance of nanocrystal-embedded microparticles was examined using the NGI system with Cyclohaler® (Fig. 4). From the deposition profile obtained from the NGI analysis, FPF value of microspheres was calculated to be in the range of 30% -39% (table 3). The microparticles containing the smallest nanoparticles exibited the higher FPF value than the other two nanocrytals-contained microparticles. All of the four types of microparticles evaluated here have the mean mass aerodynamic diameter around the range of 3.12 to 5.35 μm, which are suitable for deposition at trachea and bronchi where the main action site of BUD for the treatment of asthma and COPD.

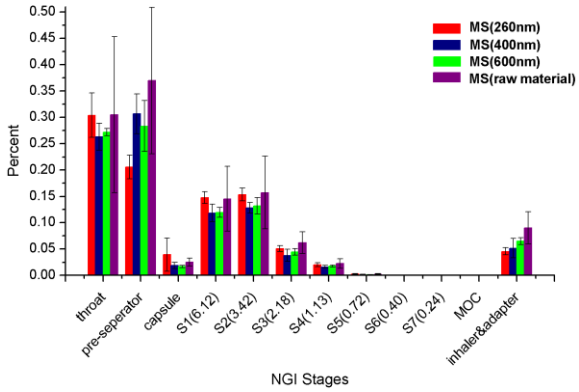


Fig.4 Aerodynamic behaviour of the microparticles containing three kinds of nanocrystals or raw material using a NGI at a flow rate (Q) of 100 L/min (Mean ± SD, n = 3).

Discussion and conclusion

In current study, BUD loaded nanoembedded HA microparticles were successfully prepared by using the technique of spray drying. In order to investigate the effects of size of BUD nanocrystal on the performance of nanocrystal-contained microspheres, three kinds of BUD nanocrystals with different particles size was prepared. Three types of nanocrystals appeared the same crystalline form with raw BUD materials and the crystalline form was maintained during the process of spray drying. Importantly, the spray dried BUD loaded nanoembedded HA microparticles exhibited relative high FPF value and suitable aerodynamic particle size for inhalation. Therefore it is interesting to see how is the performance of these microparticles with respect to in vitro release profiles, mucus retention time in vitro and PK/PD study. In addition, the impact of scaling the process up and the stability of the particles need to be assessed in the future.

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