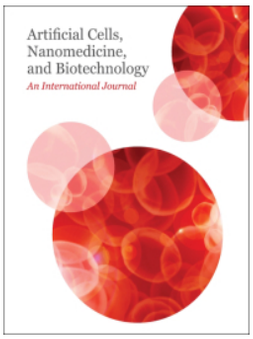

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Preparation of budesonide nanosuspensions for pulmonary delivery: Characterization, in vitro release and in vivo lung distribution studies

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Abstract

The main objective of the present article was to prepare stable and well-dispersible budesonide (BUD) nanosuspensions by microfluidizer method. The morphology, particle size, and zeta potential of formulation were investigated and in vitro release and in vivo lung distribution were evaluated. Characterizations showed that BUD nanosuspensions were spherical in shape with a smooth surface. The measured average particle size was 122.5 ± 6.3 nm, and ζ potential was − 13.6 ± 0.4 mV. In vitro release behavior of three batches BUD nanosuspensions had a good reproduction. The deposition distribution of BUD different formulations was measured using a modified multi-stage liquid collision method. The data showed that BUD nanosuspensions have the most outstanding deposition distribution with fine particle ratio 82.2%. Compared with normal particle and micronized particles, nanosuspensions were easier to be distributed in lung. After inhalation of 1 h, the drug concentration can reach 872.9 ng/g, which was extremely significantly different from normal particles ($p < 0.01$) and significantly different from micronized particles ($p < 0.05$).

Keywords: budesonide, characteristic, in vitro, in vivo, nanosuspension

Introduction

Nowadays there are nearly 200 million asthma patients worldwide and the number keeps growing every year (Spangler et al. 2013). In China, the number of patients has reached more than 10 millions in recent years (Xie and Wenzel 2013). According to reports (Persson 2014, Brightling and Desai 2013), bronchial asthma is a chronic airway inflammation, which involved a variety of inflammatory cells dominated by the eosinophils. Recurrent symptoms seriously impact the health and quality of life of patients. Clinically now, glucocorticoids are the most effective anti-allergic inflammatory drugs (Carr 2013). Its administration method is mainly anapnotherapy, which means to deliver the drugs directly to the target organ – the lung. It helps to be absorbed in the lung receptor sites. Our new Bronchial Asthma Prevention Guide and the Global Initiative for Asthma considered inhalation corticosteroids as the most effective drug formulations to control airway inflammation in asthma (Olaguibel et al. 2012, Kroegel 2009).

Currently, BUD is one of the mainly used corticosteroids clinically, which has two commercially available formulations including aerosol and dry powder inhalation (Dyer et al. 2006, Barnes 2007, Postma et al. 2008). Because of individual differences in the amount of air intake, dry powder inhalation is not as good as aerosol in terms of dose control, especially for children and elderly patients who find it more difficult to maintain a sufficient amount of the drug intake. Aerosol has good absorption effect, but its propellant contains Freon which is harmful to the environment and human bodies. In addition, since the drugs are water insoluble, when the drugs with a particle size of 0.5–7 μm enter the respiratory, some of the drugs may deposit in parts outside respiratory (mouth, throat and esophagus) leading to reduced effect of the drugs. Thus, doctors and patients both need a new kind of BUD delivery system.

Nanosuspensions is a stable colloidal dispersion of nanoparticles by using surfactant as suspending agent to spread drug kernel in water and crush or control crystallization technology (Kesisoglou and Mitra 2012, Liu et al. 2012, Chavhan et al. 2011). It is different from the traditional matrix skeleton type nanosystems. Nanosuspensions technology can reduce the drug particle size, increase the drug-specific surface area, and make the drugs easier to be absorbed in the body. Thus, it contributes to increase the bioavailability of the drug. Nanosuspensions do not need carrier material, and with the stabilization of the surfactant, it can spread nano-drug particles in water to form a stable system.

The main objective of the present article was to prepare stable and well-dispersible BUD nanosuspensions and investigate the characteristic (morphology, particle size and zeta potential), *in vitro* release, and *in vivo* evaluation by using guinea pigs model.

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Materials and methods

Materials

BUD APIs (Hangzhou Moon Fine Chemical Co., purity 99.6%, batch number 2010132); HPMC (hydroxypropyl methyl cellulose, Shanghai Colorcon Coating Technology Co., pharmaceutical grade); SLS (sodium lauryl sulfate, Shanghai chemical Reagent Co., domain research, pharmaceutical grade). All reagents for high performance liquid chromatography (HPLC) analysis, including acetonitrile and methanol were of HPLC grade. Other reagents were of analytical grade. Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment.

Preparation of nanosuspensions

HPMC (0.45 g) and SLS (0.3 g) were dissolved by stirring in an amount of water to swell overnight. After completely dissolved, 100 mg BUD were added into the solution and stirred until the final mix volume reached to 100 ml. Then the mixed solution was put in an ice bath and mixed probe sonication (220 W, 5 min, work 2 s, intermittent 1 s). After that, the mixed solution was homogenized by Nano DeBEE nano-pressure micro jet homogenizer (BEE International Company), parameters: pressure 22000 psi, the number of cycles was ten. Eventually, we get light blue opalescence BUD nanosuspensions.

Characterization

The morphological examination of the nanosuspensions was performed using a transmission electron microscope (Philips CM120, the Netherlands). In practice, a drop of BUD nanosuspensions diluted with water in room temperature condition was placed on a carbon film coated on a copper mesh and observed at 80 kV in the electron microscope. Particle size distribution and mean diameter of the prepared BUD nanosuspensions were determined by dynamic light scattering using a NICOMP 380 Submicron Particle Sizer (Santa Barbara, CA, USA) equipped with a 5-mW helium–neon laser at 632.8 nm. Sample solutions filtered through a 0.22-μm filter membrane were transferred into the light scattering cells. The intensity autocorrelation was measured at a scattering angle of 90º at room temperature. Data were analyzed in terms of intensity-weighted NICOMP distributions. Each reported experimental result was at the average of at least three d_h values obtained from analysis of the autocorrelation function accumulated for at least 20 min.

In vitro release studies

In vitro release properties of BUD from the nanosuspensions were investigated in an aqueous release medium distilled water containing 0.1 N HCl solution by a dialysis method (Daheb et al. 2013). Two milliliter of nanosuspensions were introduced into a dialysis bag (MWCO = 8–10 kDa). The end-sealed dialysis bag was immersed into 16 ml release medium at 37°C. The release medium was stirred at the speed of 60 rpm for 12 h. Samples of 0.2 ml were withdrawn at different time intervals and replaced with an equal volume of fresh release medium. The concentration of BUD in the samples was determined by the HPLC method described below.

Deposition distribution evaluation

The deposition distribution of BUD different formulations was measured using a modified multi-stage liquid collision method. Figure 1 was the schematic diagram of multi-stage liquid impinge (MSLI, Cop1ey, UK). Briefly, 0.1 ml BUD different formulations were sprayed into the MSLI. The drug with different particle sizes stay at different layers (throat and stages I–IV). The particle with diameter >10 μm sediments in the throat and stage I layer, particle with 5–10 μm diameter mainly settles in the upper respiratory tract (stage II), particle with 1–5 μm diameter mainly settles in the lower respiratory tract and lungs (stage III), and the diameter <1 μm deposition of inhaled particles settle in the lung (stage IV). After collecting all stages of the drug, the concentration of each layer containing BUD was calculated by HPLC method. The fine particle ratio (FPR) was used to evaluate the efficacy of the respiratory tract deposition distribution. FPR is defined in the formula described below; the higher the FPF, the more the particles that were smaller than 5 μm. It means that the more particles can reach the deep lung, the better effectiveness of the drugs. BUD powder and micronized particles were used as the contrast; each sample was parallel tested three times.

$$\mathrm{FPR}\% = \frac{\text{drug mass collected from stage III} \sim \text{IV}}{\text{drug mass collected from throat and stage I} \sim \text{IV}} \times 100$$

Lung distribution

Eighteen healthy Holstein guinea pigs (250–280 g, half male, purchased from Hospital Laboratory Animal Center) were used in the experiment to assess the effect of nanosuspensions on the distribution of BUD after inhale administration. The guinea pigs were divided into three groups at random

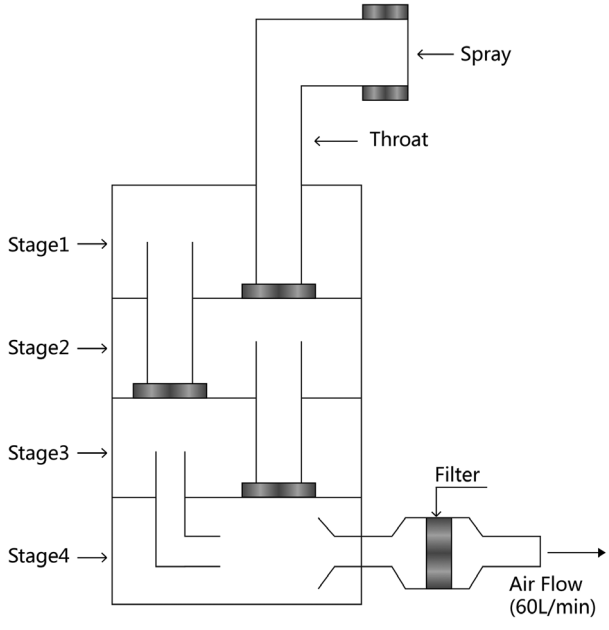

Figure 1. Schematic diagram of multi-stage liquid impinge.

and given a single 10 mg/kg dose of the BUD nanosuspensions, raw material particles or micronized particles. At 1 h after administration, each animal was euthanized, and lung samples were collected. Tissue samples were washed in ice-cold saline, blotted with paper towel to remove excess fluid, weighed and stored at −70°C until assessed for drug concentration by HPLC.

HPLC analysis

The analysis of BUD levels *in vitro* and *in vivo* were carried out using an RP-HPLC method on a system equipped with a Agilent 1260 Series and a HS2000 interface (USA Agilent) operated at 245 nm. The column was Dikma Diamonsil C18 (5 μm, 200 × 4.6 mm). The mobile phase consisted of acetonitrile:water (40:60, v/v) and the flow rate for the mobile phase was 1.0 ml/min. The column temperature was 30°C. Tissue samples were homogenized in a mixed solution of 100 mg of lung tissue and 100 μl of water. Then stirring by a homogenizer, 100 μl mixed solution was added into 5 ml tube and 300 μl of acetonitrile was added for precipitation. After centrifugation at 12000 rpm for 10 min, the clear supernatant was removed and 20 μl of sample was used for HPLC analysis.

Results

As shown in Figure 2, BUD nanosuspensions were spherical in shape with a smooth surface. The measured average particle size was 122.5 ± 6.3 nm, with a polydispersity of 0.19 ± 0.003. ζ potential was −13.6 ± 0.4 mV. The chemical and physical stability assessment for BUD nanosuspensions was conducted to support long-term storage. Fresh-

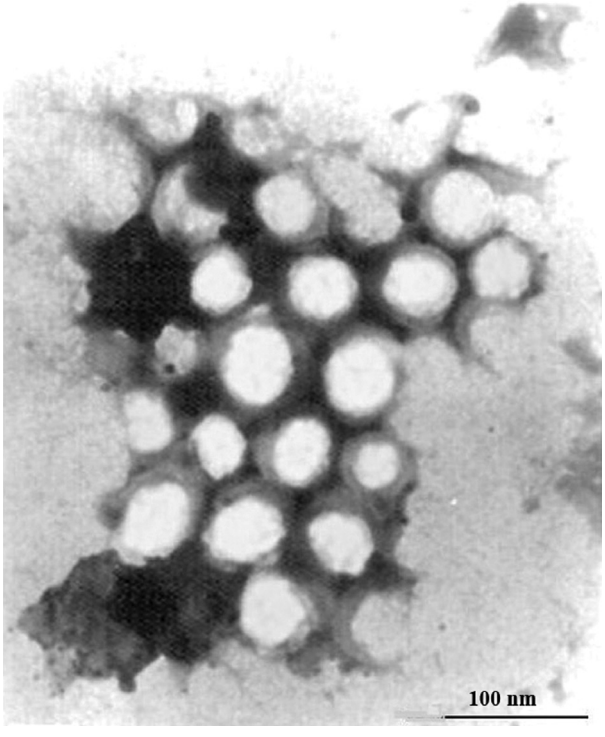

Figure 2. Transmission electron microscope photograph of budesonide nanosuspensions.

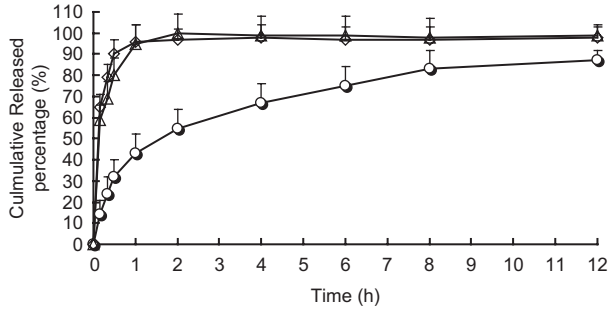

Figure 3. In vitro release profiles of budesonide nanosuspensions (o), powder (Δ) and micronization (◊) formulations ($n = 3$).

prepared formulations were stored at 25 ± 2°C for up to 12 months. Stability samples were then analyzed for physical change, drug content, particle size and zeta potential. The drug content in long-term storage conditions did not vary to a large extent in the nanosuspensions formulations; the maximum variation of 1.2% from the initial concentration was seen, 12 months from the date of manufacture. And the physical–chemical characteristics changes were found to be negligible and had no impact on the quality of the formulations. In this experiment, this release medium system could maintain a good sink condition for the *in vitro* release studies of BUD. Figure 3 showed the *in vitro* release profiles of three batches of BUD nanosuspensions. From the data, the *in vitro* release behavior had a good reproduction. In the initial 0.5 h, release was nearly linear with 10% released per day. Thereafter, a gradual slow release was observed and about 85% of the drug was released at 12 h. The *in vitro* release was kinetically analyzed according to zero-order, first-order, and the Higuchi release mechanism. The relative high correlation coefficient values obtained from the analysis of the amount of the drug released versus the square root of time indicated the release followed the Higuchi (Higuchi 1962) kinetic model, as shown in Table I.

Figure 4 and Table II showed the ratio of the value of BUD deposition distribution of different formulations. It can be seen from BUD powder group that since most of the drugs had particles larger than 10 μm, nearly half of the particles were deposited in the throat layer and could not enter the lower layer of MSLI, with FPF only 6.1%. Secondly, after the BUD micronized group, most of its particle size was maintained around 1–5 μm; therefore, it can exhibit good deposition distribution, with particle size distribution mainly in the stage III layer (36%) and FPF reached 57.5%. BUD nanosuspensions have reached the level of nanoscale, so the distribution of the particles was the most outstanding, with only 2% and 5% of the particles distributed in the throat layer and stage I layer. A large number of particles were distributed in stage IV layer (70%) and FPF was 82.2%.

Table I. Correlation coefficients for kinetic analysis of release data for budesonide nanosuspensions.

	Correlation coefficient (r)		
Formulation	Zero order	First order	Higuchi
Budesonide nanosuspensions	0.9831	0.9812	0.9993

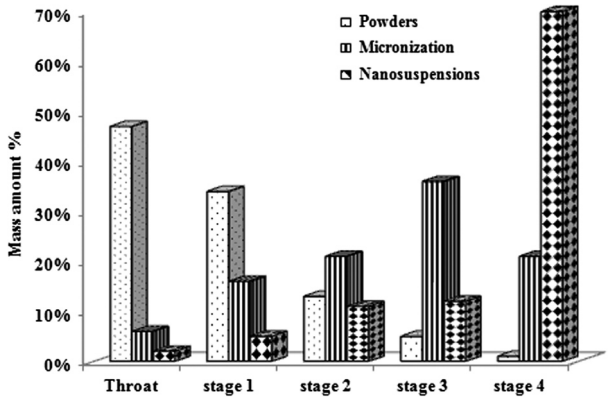

Figure 4. Dispersion behaviors of different budesonide formulations.

In vivo biodistribution behavior of BUD after inhale administration of nanosuspensions to rats was investigated with BUD powder and BUD micronized as a control. The amounts of drug distributed in unit mass of lung were measured (Table III). Compared with normal particle and micronized particles, nanosuspensions were easier to be distributed in lung. After inhalation of 1 h, the drug concentration can reach 872.9 ng/g, which was extremely significantly different from normal particles ($p < 0.01$) and significantly different from micronized particles ($p < 0.05$).

Discussion

The BUD nanosuspensions had an average size around 100 nm with a narrow polydispersity. Surface charge is an important indication of the stability of a colloidal nanoparticle system in medium. The repulsion among the nanoparticles with same type of surface charge provides extra stability. The zeta potential of the BUD nanosuspensions indicated negative charges on the nanoparticle surface (-13.6 ± 0.4 mV).

There are currently several classic methods for evaluating lung deposition of particles inhaled, one is in vitro airway simulation method, using artificial airway particle by particle size stratification to predict lung deposition model, and another method is for the in vivo distribution, direct evaluation of in vivo drug concentrations in lung tissue after administration by inhalation. This study combined the two methods to evaluate the advantage of BUD nanosuspensions.

As we all know, the size of particles is the key element that affect drug deposition in lung. Only the drug particles with diameter less than or equal to 1–5 μm can be deposited in the deep lung in order to play a pharmacodynamics. Thus, drug powder pulmonary is closely related with drug micronization technology. Many production methods are currently available for the preparation of the nanosuspensions. The study showed that the mechanical pulverization method is the most widely used pharmaceutical powder technology. However, it has disadvantages with large energy consumption, low efficiency, wide particle size distribution, and heat is not easily stabilizing drugs such as structural damage and degradation shortcomings. Supercritical technology is one of the hotspots of ultrafine particles in the preparation of 1–2 μm BUD formulations (Lobo et al. 2005, Velaga et al. 2002). However, the manufacturing process performed under high pressure and high equipment requirements (Rasenack et al. 2003) is prepared to get BUD ultrafine particles by liquid precipitation, but the resulting particles were greater than 20% of 10 μm, which did not apply to pulmonary administration. Compared with traditional high-speed mixer, ultrasonic instrument and homogenization, the new high-pressure homogenizer (HPH) can produce smaller particle size with good distribution and stability. Also, the new HPH updates rapidly. The new generation of HPH and microfluidizer has better performance with higher efficiency and higher process stability. They are also suitable for industrial production and have been widely accepted and applied to many research institutions and production enterprises.

Table II. Fine particle ratio (FPR) for three different budesonide formulations ($n = 3$).

	Formulations		
Parameter	Powders	Micronization	Nanosuspensions
FPR	6.1%	57.5%	82.2%[a,b]

Note: [a]$p < 0.05$: nanosuspensions vs. powders. [b]$p < 0.05$: nanosuspensions vs. micronization.

Table III. Budesonide concentration in lung of three different budesonide formulations ($n = 6$).

	Formulations		
Parameter	Powders	Micronization	Nanosuspensions
Budesonide concentration (ng/g)	57.4 ± 21.6	658.9 ± 62.7	872.9 ± 126.1[a,b]

Note: [a]$p < 0.01$: nanosuspensions vs. powders. [b]$p < 0.05$: nanosuspensions vs. micronization.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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