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Interest of cyclodextrins in spray-dried microparticles formulation for sustained pulmonary delivery of budesonide

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ABSTRACT

To achieve an efficient lung delivery and efficacy, both active ingredient aerosolisation properties and permeability through the lung need to be optimized. To overcome these challenges, the present studies aim to develop cyclodextrin-based spray-dried microparticles containing a therapeutic corticosteroid (budesonide) that could be used to control airway inflammation associated with asthma. The complexation between budesonide and hydroxypropyl-β-cyclodextrin (HPBCD) has been investigated. Production of inhalation powders was carried out using a bi-fluid nozzle spray dryer and was optimized based on a design of experiments. Spray-dried microparticles display a specific "deflated-ball like shape" associated with an appropriate size for inhalation. Aerodynamic assessment show that the fine particle fraction was increased compared to a classical lactose-based budesonide formulation (44.05 vs 26.24%). Moreover, the budesonide permeability out of the lung was shown to be reduced in the presence of cyclodextrin complexes. The interest of this sustained budesonide release was evaluated in a mouse model of asthma. The anti-inflammatory effect was compared to a non-complexed budesonide formulation at the same concentration and attests the higher anti-inflammatory activity reach with the cyclodextrin-based formulation. This strategy could therefore be of particular interest for improving lung targeting while decreasing systemic side effects associated with high doses of corticosteroids. In conclusion, this works reports that cyclodextrins could be used in powder for inhalation, both for their abilities to improve active ingredient aerosolisation properties and further to their dissolution in lung fluid, to decrease permeability out of the lungs leading to an optimized activity profile.

1. Introduction

Lung administration of therapeutic compounds is a challenging research field both for systemic absorption and topical therapy. Dry powder inhalers are widely used to deliver drug powder to lung patients. Traditional formulations are based on micronized drugs formulated with a carrier, typically lactose monohydrate (Healy et al., 2014). However, these formulations can be associated with issues significantly affecting drug delivery such as blend uniformity or aerosolisation properties due to little control over particle size, shape and surface morphology (Minne et al., 2008). In order to optimize the delivered dose, conventional formulations can be substituted by engineered powders containing the active ingredient (API) alone or in combination with excipients displaying various properties such as liposomes, poly(lactic-co-glycolic acid) (PLGA) or leucine (Hoppentocht et al., 2014). Some manufacturing processes are available to control the formation of such particles with specific size ranges (1–5 μm) and shapes, including supercritical fluid drying or spray-drying (Belotti et al., 2015; Buttini et al., 2012; Chow et al., 2007; Vehring, 2008). Next to an optimization of the delivered dose, efforts have been made to prevent rapid drug elimination from lung tissue (Bayard et al.,

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2013). Improving lung residence time remains a key challenge when considering high permeability in airways leading to sub-optimal pharmacokinetic profile, systemic side effects and short duration of action in the lungs (Bayard et al., 2013; Loira-Pastoriza et al., 2014). Several strategies have already been developed including promising nanoparticles, while some safety concerns still need to be addressed (Beck-Broichsitter et al., 2012). Increased lung persistence is of particular interest in various pulmonary pathologies. Among them, asthma is characterized by chronic airway inflammation. Innate and adaptive immunity effectors induce mucus overproduction, bronchial hyper-reactivity and airway wall remodelling (Lambrecht and Hammad, 2014) leading to variable levels of airflow obstruction. Inhaled corticosteroids such as budesonide are used as first-line therapy agents to reach control of airway inflammation and therefore to improve patient pulmonary functions (Holgate and Polosa, 2006). Several major parameters influence the activity of corticosteroids, including the lung residence time which is highly influenced by the release rate locally obtained. Indeed, a decrease in absorption out of the lungs will extend the contact time between corticosteroids and their receptors, thus enhancing their anti-inflammatory effect (Derendorf et al., 2006). The aim of this study is to develop a new formulation containing budesonide with (1) an improved respirable fraction and (2) a lower permeability across the lung epithelium to allow a better anti-inflammatory management. To this end, we decided to evaluate the interest of cyclodextrins as unique excipient in a powder for inhalation formulation containing budesonide. These widely known pharmaceutical excipients have already been used in the development of powders for inhalation. Indeed, they are able to induce pore formation on PLGA microparticules due to osmotic flow toward the internal phase (Loira-Pastoriza et al., 2014), leading to better aerodynamic properties. Moreover, further to the complexation of API (such as insulin), cyclodextrins are able to slow the API release from PLGA particles (De Rosa et al., 2005; Ungaro et al., 2009), leading to a sustained lung delivery. Even if not yet marketed in powder for inhalation, natural cyclodextrins are referenced in the GRAS ("generally recognized as safe") list of the FDA and hydroxypropyl-β-cyclodextrin (HPBCD) is cited in the FDA's list of inactive pharmaceutical ingredients. Thanks to their ability to form water soluble complexes with poorly water soluble drugs (Brewster and Loftsson, 2007; Loftsson and Brewster, 2010), cyclodextrins can be used to solubilize budesonide before being spray-dried to allow the formation of engineered powder for inhalation containing budesonide. HPBCD was selected considering its very low toxicity profile, including after pulmonary administration (Evrard et al., 2004). The atomization process was also optimized based on a design of experiments in order to reach both an appropriate size for inhalation and an efficient production. Next to the particle engineering, the ability of HPBCD to act as a sustained delivery system through its potential impact on budesonide lung permeability was investigated.

2. Materials and methods

2.1. Chemicals and solutions

HPBCD (Kleptose® HPB—molar substitution = 0.64) was kindly provided by Roquette (Lestrem, France). Budesonide was obtained from INDIS (Aartselaar, Belgium) and Miflonide 200® from Novartis (Basel, Switzerland). Inhalac 230® (monohydrate lactose) was kindly provided by Meggle (Wasserburg, Germany). Phosphate Buffer Saline (PBS) was provided by Lonza (Verviers, Belgium). Trimethylsilyl-3-propionide acid-*d4* (TMSP) and deuterium oxide (99.96% D) were purchased from Eurisotop (Gif-sur-Yvette, France). Phosphate buffer powder was provided by Sigma–Aldrich (Karlsruhe, Germany). Ultrapure water (18.2 MW/cm resistivity) was produced by a Milli-Q® system (Millipore). Cell culture media, supplements and Hank's Balanced Salt Solution (HBSS) were purchased from Life Technologies (Gent, Belgium), ovalbumin powder from Sigma–Aldrich (Karlsruhe, Germany) and aluminium hydroxide from Thermo Scientific (Waltham, USA).

2.2. Budesonide quantification

High performance liquid chromatography (HPLC) was used to quantify budesonide using a Merck D-7000HPLC system, Pump (L-7100), UV detector (L-7455) operating at 250 nm and with a 150/4 mm column filled 5 μm C18 (Purospher C18 endcapped). The mobile phase was composed of methanol/water ($65/35_{(v/v)}$) at a flow rate of 1 ml/min. The process was fully validated based on total error as decision criterion. The acceptance limits were set at ±5% in the range of 50–100 μg/ml and at ±10% in the range of 0.25–50 μg/ml. The minimum probability to obtain future results within these limits was set at $\beta = 95\%$ (β-expectation limits). All validation results were computed using the e-noval® software (Arlenda, Liege, Belgium).

2.3. HPBCD quantification

Proton nuclear magnetic resonance (^{1}H NMR) was used to quantify HPBCD in aqueous solution. As already described (Dufour et al., 2015), all samples were recorded at 298 K on a Bruker Avance spectrometer operating at 500.13 MHz for the proton signal acquisition. The instrument was equipped with a 5 mm TCI cryoprobe with a Z-gradient. A 1D NOESY-presat sequence was used in order to minimize the water signal. The process was fully validated based on total error as decision criterion. The acceptance limits were set at ±7.5% and the minimum probability to obtain future results within these limits was set at $\beta = 95\%$ (β-expectation limits). All validation results were computed using the e-noval® software (Arlenda, Liege, Belgium).

2.4. Phase solubility studies

Phase solubility studies were performed by adding excess amount of budesonide to aqueous solutions of HPBCD of increasing concentrations (0; 5; 10; 20; 50 and 100 mM). After four days shaking at room temperature, the undissolved budesonide was removed by filtration through a 0.22 μm filter unit (Millex-GS, Millipore) and budesonide concentrations in resulting solutions were assessed by HPLC.

2.5. NMR studies

Budesonide-HPBCD complex solutions were analyzed at 298 K on a Bruker Avance spectrometer operating at 500.13 MHz for the proton signal acquisition and equipped with a 5 mm TCI cryoprobe with a Z-gradient. Solutions were prepared by adding excess amount of budesonide to a 10 mM solution of HPBCD. After four days shaking at room temperature, the undissolved budesonide was removed by filtration through a 0.22 μm filter unit and the resulting solution was lyophilized. The solution was prepared by dissolving 10 mg in 700 μl of D_2O. Due to the nature of the samples, a presaturation sequence was used in all the experiments in order to minimize the water signal. All data were referenced to internal sodium 3-trimethylsilyl-2,2,3,3-d4-propionate (TMSP) at 0.00 ppm chemical shift (all spectra are calibrated with regard to TMSP). The ^{1}H-NMR spectra were acquired using a 1D NOESY-presat sequence. The NOESY-presat experiment used a RD-90-T1-90-tm-90-sequence with a relaxation delay of 4 s, a mixing time of 100 ms and a T1 delay of 20 s. The water suppression pulse was