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Dexamethasone palmitate large porous particles: A controlled release formulation for lung delivery of corticosteroids

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ABSTRACT

We have optimized a formulation of a prodrug of dexamethasone (DXM), dexamethasone palmitate (DXP) for pulmonary delivery as a dry powder. Formulations were prepared by spray drying DXP with 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) and Hyaluronic Acid (HA) as excipients. Large porous particles around 13 μm were produced with a tap density of 0.05 g/cm^3 and a Fine particle fraction around 40%. The palmitate moiety favors DXP insertion into DPPC bilayers therefore limiting its in vitro release as shown by differential scanning calorimetry. After administering DXP powder intratracheally to rats by insufflation, bronchoalveolar lavage fluid (BALF) and blood samples were collected up to 24 h and DXP and DXM concentrations were determined by HPLC analysis after extraction. PK parameters were evaluated according to a non-compartmental model. We observe that DXP remains for up to 6 h in the epithelial lining fluid (ELF) of the lungs at very high concentration. In addition, DXP concentration decreases according to two characteristic times. Consequently, DXM can be detected at rather important concentration in ELF up to 24 h. The passage of DXP from the lungs to the bloodstream is very poor whereas DXM seems to be absorbed in the blood more easily. These results suggest that once administered DXP undergoes two different processes: hydrolysis into DXM due to the presence of esterases in the lungs and distribution in the lung tissue. This formulation appears promising to reduce systemic exposure and prolong the effect of the drug locally.

1. Introduction

Asthma is a chronic inflammatory disorder of the airways, usually associated with airway hyper-responsiveness and variable airflow obstruction, that can be reversed either spontaneously or under treatment (Global Initiative for Asthma, 2014). Allergen sensitization is an important risk factor for asthma (Bateman et al., 2008) and for the past 40 years, the prevalence of asthma has increased in all countries in parallel with that of allergy. It is estimated that as many as 300 million people of all ages in the world suffer from asthma (Global Initiative for Asthma, 2014). Anti-asthmatic treatments can be administered via different routes of administration such as parenteral or oral (prednisone) but the pulmonary route is often preferred, as it allows delivering the medication right to the site of action where it is needed. Anti-asthmatic treatments vary depending on the extent of the disease but for quick relief they mostly consist in a combination of beta2 adrenergic agonists and corticosteroids. Beta2 adrenergic agonists such as albuterol, levalbuterol, metaproterenol or terbutaline are used as bronchodilators helping to relax airway muscles within 5 min (Spina, 2014). They lead to an increase of the airflow, facilitating patient breathing. Beta2 adrenergic agonists help relieving asthma symptoms for 3 to 6 h. Corticosteroids such as beclomethasone propionate, fluticasone furoate or propionate, budesonide, ciclesonide, or flunisolide are used for their anti-inflammatory effects and required several administrations per day. Inhaled corticosteroids are effective in the treatment of asthma because of their ability to interfere with multiple inflammatory processes involved in the asthmatic pathology (Allen et al., 2003; Barnes and Pedersen, 1993; Crim et al., 2001). Inhalation offers advantages for the treatment of asthma using corticosteroids as compared with the systemic route. It helps, with efficiency and optimal tolerance; to locally treat a condition that requires the use of high doses of active product by systemic route. The inhaled corticosteroids allow a rapid onset of action and induce fewer side effects than does administration by other routes (Beck-Broichsitter et al., 2009; Boisson et al., 2014). However, these benefits are often associated with limited lung deposition and short duration of action because of respiratory

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protection mechanisms (Hanania et al., 1995). Thus, the ideal inhaled corticosteroid should have: long residence time in the lung; intrinsic activity; low oral bioavailability and high systemic clearance resulting in negligible systemic side effects. However, most currently marketed inhaled corticosteroids still require several daily administrations (Burgt et al., 2000; Czock et al., 2005; Hübner et al., 2005). To modify corticoids residence time in the lungs, an appropriate formulation can be developed to deliver these molecules.

Lung delivery can be achieved by nebulization of drug solutions (Rodrigo, 2015) or suspensions of nanoparticles (Sahib et al., 2011), or using either metered dose inhalers(Berger et al., 2014) or dry powders for inhalation (Chawes et al., 2014). Current formulation approaches of corticosteroid dry powders consist in micronizing the active pharmaceutical ingredient and blend it with lactose as a carrier, which characteristics can be optimized (Buttini et al., 2008; Donovan and Smyth, 2010; Hoppentocht et al., 2014). Alternatively, large porous particles (LPPs), characterized by geometric sizes > 4–5 μm and mass densities lower than 0.4 g/cm^3, have been introduced for both local and systemic applications by the pulmonary route to the lungs (Cruz et al., 2011; Edwards and Dunbar, 2002; Edwards et al., 1997; Gervelas et al., 2007; Pham et al., 2015). A major advantage of LPPs relative to conventional inhaled therapeutic aerosol particles is their aerosolization efficiency (Dunbar et al., 1998; Edwards and Dunbar, 2002). This allows the supply of large drug masses using a simple inhalation device (Edwards, 2002). These characteristics suggest that inhalation of LPPs may be beneficial in the treatment of asthma by delivering the corticosteroids directly to the primary site of inflammation to achieve therapeutic local drug concentrations with low systemic exposure.

To favor prolonged efficacy of the corticosteroid it appeared interesting to combine LPPs with a corticosteroid prodrug. We have chosen to use a prodrug of dexamethasone since this corticosteroid is often used a reference to evaluate the efficacy of other molecules (Kelly, 2009). Dexamethasone palmitate is of particular interest since its aliphatic chain may help modulating its release from lipid-based LPPs. As excipients,dipalmitoyl-snglycero-3-phosphatidylcholine (DPPC) and hyaluronic acid (HA) have been chosen due to their biocompatibility, biodegradability and ability to yield porous particles (Gomez-Gaete et al., 2008). DXP encapsulation into DPPC-HA microparticles was optimized by spray drying, varying DXP concentration with respect to the lipid content. Physico-chemical characterization of the powders will be presented as well as aerosolization efficacy using a multistage liquid impiger and drug release in sink conditions. Once the formulation optimized, pharmacokinetics of DXP and its active metabolite, dexamethasone (DXM), were determined in plasma and epithelial lung fluid after intratracheal administration of the DXP powder by insufflation.

The aim of the work is therefore to optimize the formulation of DXP by spray-drying into large porous particles and to evaluate their pharmacokinetics and lung distribution in vivo in rats. We expect the delivery of a lipidic corticosteroid prodrug will allow to prolong drug residence in the lungs.

2. Materials and methods

2.1. Materials

Dexamethasone palmitate (DXP) was provided by Interchim (France), Dexamethasone (DXM) and Dexamethasone acetate (DXA) were provided by ChemosGmbH (Germany), Testosterone decanoate (TC) was provided by Sigma–Aldrich (France), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) by Corden Pharma (Switzerland) and hyaluronic acid, sodium salt 95% (HA) (MW = 1000 kDa) by Acros Organics. Acetonitrile was of high-performance liquid chromatography (HPLC) grade. All chemicals used were of analytical grade. Organic solvents were provided by Carlo Erba (Italy) and were of analytical grade when not specified. Water was purified using a RIOS/MilliQ system from Millipore (France).

Table 1
Operational conditions used for spray-drying.

Feed flow rate (mL/min)	17
Inlet temperature (°C)	150 ± 2
Outlet temperature (°C)	55 ± 4
Aspiration setting (%)	100 (35m^3/h)
Air-flow rate (L/h)	414

2.2. Microparticles preparation

DXP-loaded microparticles were prepared by spray drying using a mini spray-dryer BÜCHI B-290 (France) equipped with a 0.7 mm diameter two-fluid nozzle, which operates in a co-current mode according to conditions detailed in Table 1. An aqueous solution of HA was prepared by dissolving 200 mg of HA into 150 mL of water upon magnetic stirring at room temperature. An ethanolic solution was prepared by dissolving DPPC and DXP into 350 mL of ethanol absolute to obtain a final amount of 800 mg of lipophilic compounds. The weight percent of DXP was varied between 0 and 15%. Ethanolic and aqueous solutions were then mixed at a ratio of 70/30 (v/v) prior to spray-drying and the mixture maintained under moderate stirring while fed into the spray-dryer. The yield was calculated as a percentage of the mass of the powder collected divided by the initial mass of solids in the solution prior to spray-drying.

2.3. Particle size distribution

Powders size distributions were measured by light diffraction using a Mastersizer 2000 equipped with a Scirocco dry disperser (Malvern Instruments, France) at a dispersing pressure of 3 bars. The refractive index used was 1.5. Data obtained were expressed in terms of the particle diameter at 10%, 50% and 90% of the volume distribution (D10, D50 and D90 respectively). The span of the volume distribution, a measure of the width of the distribution relative to the median diameter was calculated according to Eq. (1). A large span is indicative of a more heterogeneous size distribution. Values presented are the average of at least 3 determinations.

$$Span = \frac{D90 - D10}{D50} \quad (1)$$

2.4. Tap density and aerodynamic diameter

Powder tap density (ρ) was determined using a tapping apparatus (Pharma test PT-TD1). Accurately weighed powder samples were filled into a 5 mL graduated cylinder and the height measured following 1000 taps which allowed the density to plateau (Pharmacopoeia, 2013). Assuming an efficient packing, the tap density of monodisperse spheres is approximately a 21% underestimate of the true particle density due to the void spaces between particles. Although polydispersity may reduce the void volume between particles, this is probably counterbalanced by an imperfect packing (Vanbever et al., 1999). Measurements were performed in duplicate.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) was performed using a LEO1530 microscope (LEO Electron Microscopy Inc., Thornwood, USA) operating between 1 and 3 kV with a filament current of about 0.5 mA. Powder samples were deposited on carbon conductive double-sided tape (Euromedex, France) and were coated with a palladium–platinum layer of about 4 nm using a Cressington sputter-coater 208HR with a rotary planetary-tilt stage, equipped with a MTM-20 thickness