European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 604–613

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Steroid/mucokinetic hybrid nanoporous microparticles for pulmonary drug delivery

Frederic Tewes [a,b], Krzysztof J. Paluch [a], Lidia Tajber [a], Karan Gulati [a], Devesh Kalantri [a], Carsten Ehrhardt [a], Anne Marie Healy [a,*]

[a] School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Panoz Institute, Dublin, Ireland
[b] INSERM U 1070, Pôle Biologie-Santé, Faculté de Médecine & Pharmacie, Université de Poitiers, Poitiers, France

ARTICLE INFO

Article history:
Received 14 September 2012
Accepted in revised form 16 March 2013
Available online 4 April 2013

Keywords:
Budesonide
Ambroxol
Nanoporous microparticles
Calu-3 cells
Andersen cascade impactor
Dry powder inhaler

ABSTRACT

In a number of pulmonary diseases, patients may develop abnormally viscous mucus reducing drug efficacy. To increase budesonide diffusion within lung fluid, we developed nanoporous microparticles (NPMPs) composed of budesonide and a mucokinetic, ambroxol hydrochloride, to be inhaled as a dry powder. Budesonide/ambroxol-HCl particles were formulated by spray drying and characterised by various physicochemicals methods. Aerodynamic properties were evaluated using a cascade impactor. Drugs apparent permeability coefficients were calculated across mucus producing Calu-3 cell monolayers cultivated at an air–liquid interface. Microparticles made only from budesonide and ambroxol-HCl had smooth surfaces. In the presence of ammonium carbonate ($(NH_4)_2CO_3$), NPMPs were formulated, with significantly ($P < 0.05$) superior aerodynamic properties (MMAD = 1.87 ± 0.22 μm and FPF = 84.0 ± 2.6%). The formation of nanopores and the increase in the specific surface area in the presence of $(NH_4)_2CO_3$ were mainly attributed to the neutralisation of ambroxol-HCl to form ambroxol base. Thus, ambroxol base could behave in the same manner as budesonide and prompt nanoprecipitation when spray dried from an ethanol/water mix occurs. All formulations were amorphous, which should enhance dissolution rate and diffusion through lung fluid. These NPMPs were able to improve budesonide permeability across mucus producing Calu-3 cell monolayers ($P < 0.05$) suggesting that they should be able to enhance budesonide diffusion in the lungs through viscous mucus.

1. Introduction

Pharmacotherapy of lung diseases often involves treatment with more than one group of therapeutic agents (e.g., bronchodilators, corticosteroids, cromoglycate, and antibiotics). In addition to the oral route, these agents are often delivered by pulmonary inhalation, in order to directly target the lung and increase the therapeutic/systemic side effect index.

The local delivery of drugs to the lung can be obtained by using dry powder inhalers (DPIs), metered dose inhalers (MDIs) or nebulisers. Whenever it is possible, the use of DPIs or MDIs is preferred by patients due to the convenience of use [1,2]. The simplest formulation and least problematic, from the point of view of stability, appears to be dry powders due to the presence of the active pharmaceutical ingredient (API) as solid particles and the lack of propellant, in contrast to pressurised MDIs, where the API is dissolved or suspended in the liquefied vehicle [3]. Also, in order to improve the results of pharmacotherapy, increase patient compliance and reduce the time of administration of several APIs, co-formulation of drugs into one product [4–7] or co-administration of drugs as a mix of solutions for nebulisation is popular. However, the latter can bring the risk of physicochemical instability of components when mixed extemporaneously [8].

To optimise the efficacy of DPIs, conventional micronised API carrier-based formulations may be substituted by an optimised API powder having specific particle morphology and designed to provide advantageous flow and aerodynamic properties. For example, tobramycin powder was produced using emulsion-based PulmoSphere technology, producing highly dispersible porous particles [9]. Excipient-free nanoporous microparticles (NPMPs) [10] of budesonide (BUD) [11] and sodium cromoglycate [12] prepared by a novel spray drying process had improved *in vitro* deposition properties compared to non-porous particles, though it remained unknown, if it is possible to produce NPMPs composed of two APIs and still be able to maintain the superior aerodynamic properties.

BUD was previously reported to form NPMPs when processed by spray drying [11]. BUD is a glucocorticosteroid which, when inhaled, has a dose-dependent anti-inflammatory action in the lung

* Corresponding author. School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Panoz Institute, Dublin, Ireland. Tel.: +353 1 896 1444.
E-mail address: healyam@tcd.ie (A.M. Healy).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2013.03.020

[13]. It acts by binding to glucocorticoid receptors in lung cell cytoplasm [14]. Budesonide has been shown to be very effective against the characteristic inflammation of asthma [15]. It is reported to reduce the risk of Chronic Obstructive Pulmonary Disease (COPD) exacerbations in symptomatic COPD patients [16]. Budesonide also resulted in an improvement in bronchial hyper-responsiveness and a decrease in cough in cystic fibrosis patient [17]. However, in these two last diseases, efficacy of inhaled corticosteroids can be strongly reduced because of the low diffusion through the viscous surface secretions of the lung [18]. In fact, in a number of pulmonary diseases, patients may develop abnormally high viscous thick mucus which is responsible for low diffusion of drugs in the lungs [5,19,20].

To reduce the viscosity of the lung fluid, mucokinetics such as ambroxol hydrochloride (AMB-HCl) are used [21]. Ambroxol may provide suitable rheological properties of the airway secretion by acting directly on ciliated epithelial cells [22]. Ren et al. [23] showed that the rat lung epithelial lining fluid (ELF)/plasma $AUC_{0\rightarrow 10h}$ ratios obtained for ambroxol hydrochloride (AMB-HCl) after dry powder pulmonary administration were 33–56 times higher compared to those achieved by intravenous administration. Thus, the co-administration of BUD together with AMB-HCl could possibly improve the diffusion of BUD in the lining fluid of the lung and enhance its bioavailability. For that reason, we developed a combined BUD AMB-HCl formulation as excipient-free NPMPs to be used in DPIs.

2. Materials and methods

2.1. Materials

Budesonide (BUD) was purchased from Tianjin Tian Mao Technology Development Corp. Ltd., China. Acetonitrile (HPLC grade), ambroxol hydrochloride (AMB-HCl) and ammonium carbonate $(NH_4)_2CO_3$ (analytical grade) were purchased from Sigma–Aldrich (Dublin, Ireland). Ultrapure water was produced by a Synergy Ultrapure Water System linked to an Elix Advantage system (Millipore, Carrigtwohill, Ireland). Sodium pyruvate, foetal calf serum (FCS), non-essential amino acids solution (NEAA), minimum essential medium (MEM), glucose, penicillin/streptomycin solution, NaCl, KCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $NaHCO_3$ and HEPES were cell culture grade and purchased from Sigma–Aldrich.

2.2. Methods

2.2.1. Spray drying

Spray drying experiments were performed based on methods described by Nolan et al. [11]. Briefly, 1% (w/w) solution of different BUD/AMB-HCl ratios, with and without $(NH_4)_2CO_3$ (Table 1), was spray dried using a Büchi B-290 Mini spray dryer (Flawil, Switzerland) from a solvent made of 80% (v/v) ethanol and 20% (v/v) water. The spray dryer was set in an open suction mode using a standard two-fluid nozzle. In all cases, the inlet temperature was 78 °C; feeding pump was set at 30% (8 mL/min); spraying gas nozzle flow rate was 15 L/min; and a flow rate of 630 NL/h was used for the drying gas (aspirator rate 100%). These conditions resulted in outlet temperatures ranging from 42 to 52 °C.

2.2.2. Scanning electron microscopy (SEM)

Scanning electron micrographs of powder samples were taken using a Tescan Mira XMU (Brno, Czech Republic) SEM. The dry powder samples were fixed on aluminium stubs with double-sided adhesive tape and a 10 nm-thick gold film was sputter coated on the samples, before visualisation. Primary electrons were accelerated under a voltage of 5 kV. Images were formed from the collection of secondary electrons.

2.2.3. Specific surface area

The specific surface area of the samples was determined by the N_2 adsorption B.E.T. multipoint method, with 6 points in the relative pressure range of 0.1–0.3, using a Micromeritics Gemini 2835c (SMS Ltd., London, UK) as previously described [11]. Samples were prepared by purging under N_2 overnight at 40 °C.

2.2.4. Particle size distribution analysis

The geometric particle size distributions (PSDs) were determined by laser diffraction using a Malvern Mastersizer 2000 (Malvern Instruments Ltd. Worcestershire, UK) with the Scirocco 2000 dry powder feeder to disperse the particles. The dispersive air pressure used was 3 bar and vibration feed rate was set to 50%. The PSD of each sample was determined in triplicate.

2.2.5. Differential scanning calorimetry (DSC)

DSC was performed using closed 40 μL-aluminium pans with three vent holes on samples (4–8 mg) weighed with a MT5 balance (Mettler Toledo, Zürich, Switzerland) [24]. Samples were run at a heating rate of 10 °C/min under nitrogen purge from 40 °C to 270 °C using a Mettler Toledo DSC 821^e (Switzerland). Mettler Toledo STAR^e software was used for analysis of thermal events.

2.2.6. Powder X-ray Diffraction (XRD)

X-ray powder diffraction measurements were conducted on samples placed in a low background silicon holder, using a Miniflex II desktop X-ray diffractometer (Rigaku, Tokyo, Japan) with a Bragg–Brentano geometry [24,25]. The samples were scanned over a range of 5–40° 2θ at a step size of 0.05° per second. The X-ray tube composed of copper anode was operated under a voltage of 30 kV and a current of 15 mA, which, after crossing a monochromator, produced copper Kα radiation (λ = 1.542 Å).

2.2.7. Drug quantification

A HPLC method, based on the method described by Nolan et al. [11], was set up to assay both BUD and AMB-HCl simultaneously. The assay was performed using a LC module I plus (Waters, UK) chromatographic system equipped with a C18, 15–20 μm, 3.9 × 300 mm apolar column (μBondapak™, Waters, Ireland). Drug elution was detected by measuring the absorbance at 242 nm. Measurements were conducted at room temperature by injecting 50 μL of samples, standards or controls in the mobile phase running at 1.5 mL/min and composed of 55% by volume of 0.05 M CH_3-COOH/Na buffer (pH 4) and 45% of acetonitrile. The running time of the assay was 7 min. A calibration curve for each drug was constructed, allowing the drug concentrations in samples to be calculated. The concentration of the standard solutions of each drug used to construct the calibration curves were in the range 6 μg/mL to 100 μg/mL.

2.2.8. In vitro deposition studies using the Andersen Cascade Impactor (ACI)

A dry powder inhaler (Cyclohaler®, N.V. Medicopharma, Zaandam, Netherlands) was filled with a size 3 hard gelatin capsule loaded with 20 ± 2 mg of powder for each test. After inhaler actuation, particle deposition in the device, the induction port, all the stages and the filter was determined by the HPLC assay described above (n = 3). A flow rate of 60 L/min was used in the ACI. The time of aspiration was adjusted to obtain 4 L. The total amount of particles with aerodynamic diameters smaller than 5.0 μm was calculated by interpolation from the inverse of the standard normal cumulative mass (mg) distribution less than stated size cut-off against the natural logarithm of the effective cut-off diameter