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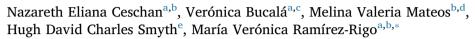
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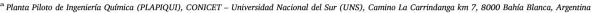
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Carrier free indomethacin microparticles for dry powder inhalation





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ABSTRACT

The present studies were designed to evaluate inhalatory microparticles carrying indomethacin (IN) for potential local (specific and non-specific bronchial inflammatory asthma responses) and systemic treatments (joint inflammation, rheumatoid arthritis and osteoarthritis pain) by optimizing microparticle properties, characterizing their lung deposition, drug release, evaluating cytotoxicity and also pharmacological effect in vitro. The acidic groups of IN were complexed with the cationic groups of the polyelectrolyte polylysine in order to increase the drug water compatibility. The polylysine/indomethacin ratio was fixed and the pH was adjusted in different formulations. Microparticles were obtained by spray drying using a relatively high atomization air flowrate (742 L/min) and a high-performance cyclone in order to optimize the production of microparticles with adequate attributes for inhalatory delivery. The produced microparticles exhibited high process yield and IN loading, volumetric mean diameters smaller than 5 µm and narrow particle size distributions. According to demonstrated aerosolization performance, the powders were suitable for inhalatory indomethacin local and systemic treatments. Emitted fraction was higher than 90%, the MMAD was around 3 µm and the GSD lower than 3. The respirable fraction for particles with aerodynamic diameters smaller than $5\,\mu m$ was around 29% while for particles with aerodynamic diameters smaller than 3 µm the value was around 17%. The addition of lactose as carrier worsened the aerodynamic performance of the microparticles. The developed powdered systems got wet and dissolved quickly and presented higher release rates respect to pure IN in simulated lung physiological conditions. Furthermore, the assays performed in RAW 264.7 cell line showed that the microparticles exhibited the same anti-inflammatory capability as the pure drug. The developed particles did not affect the RAW 264.7 cell viability. In conclusion, a promising powder formulation for DPIs has been developed to treat, locally and systemically, inflammatory diseases.

1. Introduction

Indomethacin (IN) is a non-steroidal anti-inflammatory drug (NSAID) derived from indole-acetic acid. IN is currently approved for the treatment of rheumatoid arthritis, osteoarthritis pain (El-Badry et al., 2009) and demonstrated to be useful for treating specific and non-specific bronchial inflammatory asthma responses, as well as other inflammatory pulmonary and non-pulmonary conditions (Bianco, 2000). However, oral administration of the required IN dose to achieve therapeutic concentrations in the lung is limited due to the high incidence of adverse gastrointestinal effects (Bianco, 2000). Rainsford

reported that the gastrointestinal side effects caused by the use of indomethacin accounted for 19–39% of all reported side effects (Rainsford, 1982). These adverse effects lead to treatment discontinuation (Romano et al., 2004).

For this reason, alternative administration routes have been explored for indomethacin, including percutaneous (Ricci et al., 2005), nasal (Karasulu et al., 2008) and pulmonary route (Onischuk et al., 2008). For percutaneous and nasal administration, the addition of permeation enhancers was found to be necessary to achieve adequate systemic concentrations of IN (Ricci et al., 2005; Karasulu et al., 2008). Concerning pulmonary administration, nanoparticles containing pure

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IN were demonstrated to have adequate systemic drug absorption, without the need of permeation promoters (Onischuk et al., 2008). In previous studies, a relatively complex vaporization/condensation technology was used to produce inhalation particles, and presented limited lung deposition (due to their small sizes). Separately, the US6051566 patent proposed the use of indomethacin via jet nebulization (20 mg/dose) for local treatments in the lung. The formulation and the administration technique successfully allowed the therapy of specific and non-specific bronchial stimuli in asthmatic patients, without presenting adverse effects (Bianco, 2000). Although these contributions are valuable, a Dry Powder Inhalatory system (DPI) to administer IN which provides high dose deposition in the alveolar region and fast drug dissolution rate has not yet been achieved.

In this work, microparticles containing IN were developed to administrate this drug to the airways using a DPI. DPIs have some competitive advantages respect to others inhalatory system and fulfill most of patients and physicians preference. In fact, they are less time-consuming than nebulizers and are easier to use than Metered Dose Inhalers (MDIs) because they are breath-actuated. Also, the DPIs offer high physical, chemical and microbiological stability (Geller, 2005; Roche et al., 2017). The DPI performance is a combination of the particulate-system physicochemical properties, the inhaler design and the physical mechanisms that aerosolize, deagglomerate, disperse and deposit the particles in the lung (Islam and Cleary, 2012).

The use of co-processed materials could greatly improve dry powder formulation performance. For example, biodegradable polymers have been used for a) mucoadhesion capacity, i.e. increase of the formulation residence time in lung, or in other terms, decrease the number of daily administrations (Gallo et al., 2017); b) deposition at specific pulmonary target sites (e.g., macrophages or cancerous cells) (Martinelli et al., 2016); c) modified release for the treatment of pulmonary infections (Rivera et al., 2004); d) increased penetration of drugs into target cells (e.g., increased transfection and incorporation of DNA and oligonucleotides in gene therapy) (Colonna et al., 2008), among others. Of particular interest are co-processed materials comprising a polyelectrolyte and an oppositely charged drug to allow the engineering of materials and particles with properties different from the raw materials (Ceschan et al., 2016, 2015, 2014). Particularly, viscous anionic feeds carrying a water soluble drug that led to swellable particulate systems were studied (Ceschan et al., 2016). In order to complement these results, in this article a polyelectrolyte-drug system based on a non-viscous polycation and a low water soluble drug is described.

In a previous work, different polylysine-IN formulations were processed by spray drying to obtain microparticles for inhalatory administration. Among all the studied formulations, a particulate system with relatively high IN load, appropriate aerodynamic particle size (between 1 and 5 µm) and low moisture content emerged as an optimal formulation design within those tested. Using a low-resistance inhaler, the aerodynamic performance was demonstrated to have sufficient efficiency (Ceschan et al., 2015). Based on this foundation, we hypothesized that modulating the spray drying process parameters (operating variables and fed composition) could lead to optimized indomethacin powders for inhalation. In particular, it has been demonstrated that cationic free groups can damage cell membrane (Nafee et al., 2009) and affect respiratory physiology (Gu et al., 2006). In this work the spray drying parameters such as feed composition (by adjusting the pH), the cyclone type and the atomization air flow rate were modified to develop a physiologically compatible inhalation powder to be administrated by a DPI device, either for local or systemic treatments. Powders were also mixed with lactose as an aerodynamic carrier. The developed products were also subjected to in vitro biopharmaceutical assays: cell viability, aerosolization, permeation and anti-inflammatory activity tests to study the influence of the pH adjustment and the lactose addition on these experiments.

2. Materials and methods

2.1. Materials

Indomethacin (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), epsilon-polylysine and dextrin (food grade, Purac America, Lincolnshire, United States), hydrochloric acid (analytical grade, Anedra, Buenos Aires, Argentina), lactose monohydrate 140-70 ASTM Mesh (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), potassium phosphate monobasic (analytical grade, Anedra, Buenos Aires, Argentina), sodium hydroxide (analytical grade, Anedra, Buenos Aires, Argentina), size 3 gelatine capsules (pharmaceutical grade, Parafarm, Saporiti, Buenos Aires, Argentina), glycerin (pharmaceutical grade, Anedra, Buenos Aires, Argentina) were used.

The polylysine used in this work was epsilon-polylysine which was supplied as a mixture of polylysine:dextrin (PL:DX 1:1). Dextrin allows improving flow properties (Lee et al., 2001) and storage stability (Huybrechts, 2006). This compound, which is highly water soluble, was previously used as biocompatible excipient in pulmonary formulations (Alsaadi et al., 2012). Its association with polylysine was proposed to improve the PL conformational structure stability (Huybrechts, 2006). PL is a cationic polyelectrolyte with the capability to interact with acidic compounds, like IN.

For the assays in cell cultures, mouse macrophage cell line RAW 264.7 (ATCC® TIB-71®), Dulbecco's Modified Eagle's Medium (DMEM) and antibiotic-antimycotic (Anti-Anti 100X) were provided by Gibco (Life Technologies, United States). Fetal bovine serum (FBS) was from Natocor (Córdoba, Argentina). Polyvinylidene fluoride (PVDF) membranes were obtained from Millipore (Bedford, United States). UltraCruz# Autoradiography, polyclonal horse radish peroxidase (HRP)-conjugated goat anti-rabbit IgG, polyclonal HRP-conjugated goat anti-mouse IgG were obtained from Santa Cruz Biotechnology, Inc. (California, United States). Mouse monoclonal anti-α Tubulin (DM1-A) was from EMD/Biosciences (San Diego, United States). Rabbit polyclonal antibody anti- cyclooxygenase-2 (COX-2) was from Cayman Chemical (Michigan, United States). Bovine serum albumin (BSA), Klebsiella pneumoniae lipopolysaccharide (LPS), dimethyl sulfoxide (DMSO), RIPA lyses buffer [10 mM Tris-HCl (pH 7.4), 15 mM NaCl, 1% Triton X-100, 5 mM NaF, 1 mM Na₂VO₄ and the complete protease inhibitor cocktail], TTBS buffer [20 mM Tris-HCl (pH 7.4), 100 mM NaCl and 0.1% (w/v) Tween 20] were obtained from Sigma-Aldrich (Saint Luis, United States). All these reagents were analytical grade. Distilled water was also employed.

2.2. Methods

2.2.1. Liquid feed preparation and spray drying (SD)

In previous work, liquid formulations (fed to the spray drier) with different PL:DX/IN ratios were studied to identify the influence of the feed composition on the powder properties (Ceschan et al., 2015). Among all the formulations studied in that contribution, those with a 50% of neutralization degree of the PL amino groups emerged as the most promising and thus attractive for further characterization, as they exhibited high process yield, low residual moisture and small particle size. In this work, based on the best powder presented by Ceschan et al. (2015), some selected process parameters were modified to obtain improved powders with enhanced properties (adequate particle size for systemic treatments and physiologically compatibility with cell tissues). To this purpose, two liquid formulations were prepared: a) the best formulation found by Ceschan et al. (2015), which was named (PL IN)50:DX (where the subscript 50 represents the degree of neutralization of the PL amino groups by indomethacin) and b) a formulation with the same composition than a), for which the solution pH was adjusted to almost 7 by adding HCl (denoted as (PL IN)50:DXCl).

The composition of the spray-dried solutions is detailed in Table 1. In all cases, the sprayed volume was 200 mL. The Table 1 includes the

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