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Review

Inhaled proteins: Challenges and perspectives

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ABSTRACT

Due to recent developments in biochemical engineering and in the understanding of the physiopathology of many diseases, therapeutic biologics are expected to become of increasing importance. Pulmonary delivery of these proteins could constitute an attractive, non-invasive alternative to parenteral delivery. It can be considered for either topical use for treating lung diseases or for systemic use for treating a variety of other diseases. However, administration of proteins to the lungs presents several challenges such as the need for appropriate formulation strategies to overcome high inter-particle interactions and physico-chemical degradation that can lead to loss of biological activity and/or safety issues. In addition, various lung clearance mechanisms have to be avoided to provide a sufficient level of intact protein in the lungs. If systemic action is desired, it is also necessary for the molecule to cross the alveolar epithelium, which is particularly challenging for large proteins with many hydrophilic domains.

The purpose of this article is to review the main challenges in the formulation of proteins for inhalation and the possible strategies that can be applied. Because of the particular success of dry formulations in stabilising proteins, there is a special focus on their development, along with the drying techniques and stabilising excipients used. Finally, an overview is given of the existing commercial preparations and of the main clinical developments in inhaled proteins for either topical or systemic applications.

Contents

Abbreviations: ARDS, acute respiratory distress syndrome; Asn, asparagine; BSA, bovine serum albumin; CD, cyclodextrin; COPD, chronic obstructive pulmonary disease; d_{ae}, diameter of the particles; DPI, dry powder inhaler; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoyl phosphatidylcholine; EPO, erythropoietin; FPD, fine particle dose; FPF, fine particle fraction; G-SCF, granulocyte colony-stimulation factor; GLP-1, glucagon-like peptide-1; GM-CSF, granulocyte macrophage colony-stimulating factor; GRAS, generally recognised as safe; HA, hemagglutinin; hGH, human growth hormone; ICS, inhaled corticosteroids; IFN, interferon; Ig, immunoglobulins; IL, interleukin; i.m., intramuscular; i.v., intravenous; LDH, lactate dehydrogenase; MAbs, monoclonal antibodies; MMAD, mass median aerodynamic diameter; MW, molecular weight; PEG, polyethylene glycol; PLGA, poly(lactide-co-glycolide acid); pMDI, pressurised metered dose inhaler; PTH, parathyroid hormone; rHA, recombinant human albumin; s.c., subcutaneous; SCF, supercritical fluid; T_g, glass transition temperature; TNF-α, tumour necrosis factor-α; VIP, vasoactive intestinal peptide.

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1. Introduction

A number of therapeutic proteins have been used for long in clinical practice. These include insulin to treat diabetes, human growth hormone (hGH) to supplement hormone deficiency, and calcitonine and parathyroid hormone (PTH) to treat osteoporosis, as well as protein antigens in vaccine formulations. Due to recent developments in biochemical engineering and in the understanding of the physiopathology of many diseases, therapeutic biologicals are expected to become of increasing importance. For example, novel biotherapeutical molecules have recently been developed such as monoclonal antibodies (MAbs), antibody fragments, soluble receptors, and receptor agonists or antagonists. These are mainly used to treat auto-immune and inflammatory diseases (asthma, rheumatoid arthritis) and to treat cancers (Antosova et al., 2009). However, a major drawback of these biomolecules is the need to use parenteral administration. This is mainly due to the presence of proteases and harsh pH conditions in the gastro-intestinal tract, leading to various physico-chemical degradations and loss of biological activity (Siekmeier and Scheuch, 2009).

Pulmonary delivery of these proteins could constitute an alternative to parenteral delivery. Indeed, direct administration to the lungs for local treatment allows high doses of protein drugs to be delivered while limiting systemic side effects (Dolovich and Dhand, 2011). Moreover, pulmonary delivery can be used to provide systemic action of some proteins. Due to the very high surface area of the lungs, the low thickness of the alveolar epithelium, and the high level of lung vascularisation, pulmonary administration can provide fast systemic absorption while avoiding the degradation mechanisms of the gastro-intestinal tract and the hepatic first-pass metabolism (Agu et al., 2001).

However, administration of proteins to the lungs presents some challenges. A premilinary issue with inhaled drugs, and consequently with inhaled proteins, is the need to provide the drug as very small solid or liquid microparticles (1–5 μm) to reach the lungs (Carvalho et al., 2011). For solid microparticles, this entails overcoming their very high number of inter-particle interactions by using appropriate formulation strategies and by including deaggregation mechanisms in the inhalation device. Another issue, which is specific to inhaled proteins, is that they can undergo physico-chemical degradation, which can lead to loss of biological activity and/or safety issues. Physico-chemical degradation can occur during production of the protein, which is generally achieved by the technology of recombinant DNA, using yeasts (mainly *Saccharomyces cerevisiae*) or bacteria (mainly *Escherichia coli*) as an expression host (Huang et al., 2010). Degradation is indeed frequent during fermentation, but also occurs in the extraction and purification steps. These involve relatively harsh procedures to remove completely contamination with host proteins or cell culture media, which can lead to toxicity or antigenicity issues. On the other hand, overexpressed proteins are often produced in the form of inclusion bodies, which require complicated denaturation and refolding processes to make them functional. These processes also risk causing protein degradation (Choi et al., 2006; Wang et al., 2010). Physico-chemical degradation can also occur during processing/formulation of the protein due to various stresses, such as exposure to extreme temperatures or pH, shear stress, surface adsorption, etc. (Ohtake et al., 2011). Another issue with proteins is that they are prone to biological degradation *in vivo*, due to the presence of proteases in the lungs and various clearance mechanisms in the respiratory tract and the blood (Høiby et al., 2010). Appropriate clinical efficacy of the drug can only be achieved if these clearance mechanisms can be avoided. Finally, if systemic action of the protein is required, an additionl issue is the need to cross the alveolar epithelium, which is particularly challenging because of the large molecular weight (MW) and hydrophilicity of proteins (Hussain et al., 2004).

In this review, a quick reminder is first presented of the fundamental requirements in pulmonary delivery, which must be taken into account not only for proteins but for every drug formulation. The main challenges in the formulation of proteins for inhalation are then described along with the possible strategies that can be applied. Dry formulations are particularly successful in stabilising proteins. A special focus is therefore on the development of this formulation strategy, with the drying techniques and stabilising excipients used. Finally, an overview is given of the existing commercial preparations and of the main clinical developments in inhaled proteins for either topical or systemic applications.

2. Fundamental requirements of pulmonary delivery

Particle size is one of the most important variables to design in an aerosol formulation. The inhaled particles are deposited at the different levels of the respiratory tract based on their behaviour in airflow, which depends on the size, density, and shape of particles and is characterised by the aerodynamic diameter of the particles (d_{ae}) (Telko and Hickey, 2005). In the respiratory tract, particles with an aerodynamic diameter larger than 5 μm are mainly deposited by inertial impaction in the upper airways (i.e., extrathoracic and large conducting airways), principally at or near airway bifurcations, where flow velocities are high and change direction sharply. Particles of between 1 and 5 μm are mainly deposited by sedimentation in the lower respiratory tract (i.e., bronchial tree and alveoli), where the air velocity progressively decreases. To reach the alveolus tissue specifically and therefore obtain systemic absorption, the particles need to be in the range of 1–3 μm. Deposition of these particles increases with longer residence time but decreases as the breathing rate increases (Martonen and Yang, 1996). Below the size of 0.5 μm, Brownian motion characterises the displacement of particles, which may then result in particle deposition by diffusion, especially in small airways and alveoli. However, particles of this size are mostly exhaled by the expiratory airflow. Therefore, to reach the lower respiratory tract and optimise pulmonary drug deposition, aerosols must have aerodynamic diameters between 0.5 and 5 μm (Elversson et al., 2003).

Experimental determination of aerodynamic diameters is generally performed using impaction techniques. The particle size distribution of a formulation is then mainly characterised by its mass median aerodynamic diameter (MMAD), the fine particle dose (FPD), and the fine particle fraction (FPF). The FPD is the mass of particles that have an aerodynamic diameter below 5 μm, and are therefore expected to reach lungs. The FPF is the fraction of the total drug dose with a particle size below 5 μm (Dunbar et al., 1998).

The d_{ae} can be decreased by decreasing the particle size, decreasing particle density or increasing the dynamic shape factor