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Dry powder inhalation of biopharmaceuticals

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CHAPTER 9

Summary

The rapid developments in molecular biology and biotechnology during the past decades have resulted in the successful introduction of a number of biopharmaceuticals (among which many therapeutic peptides and proteins) on the market. Biopharmaceuticals are a special class of medicines in the sense that, in general, they are chemically and physically not stable. In addition, biopharmaceuticals rely on a certain (three dimensional) folding structure for their efficacy and safety. Retaining the folding structure is essential to develop efficacious and safe drug products of these substances.

Of all biopharmaceuticals, a number may well be suited for inhalation. The objective of the inhalation can be to exert either a local or systemic action, or both. Various device types are available for inhalation. The aerosol can be created from liquids or dry powders. Nebulization is not patient friendly; it is time consuming, requires electricity or pressurized air and the efficacy of aerosolisation and lung deposition are poor which is associated with unnecessary wastages. On the other hand, dry powder inhalation typically needs less than a minute for completion and the efficiency of aerosol production and lung deposition are relatively high.

As biopharmaceuticals are often manufactured in solutions, a drying step is required to make them suitable for powder inhalation. Especially those biopharmaceuticals that depend on their tertiary structure for activity, require stabilization during drying and storage. Excipients like sugars seem suitable for this purpose. They immobilize the molecules and offer the possibility to replace the hydrogen bonds between drug and solvent.

Several drying methods are available and a number of these methods have been investigated in this thesis: freeze drying, spray-freeze drying and spray drying. Other techniques like supercritical fluid extraction may be suitable too. Spray-freeze drying gives a solid powder as end-product. By appropriate settings of the process conditions a powder with the desired aerodynamic particle size may be obtained. Spray drying also provides the possibility to produce a powder within the desired size range.

In this thesis we investigated the formulation of several peptides and proteins as powder for inhalation. Furthermore, a “proof-of-concept” study was performed with several products. We examined a selection of peptides and proteins, with different properties in terms of hydrophilicity and molecular size. Finally an innovative concept, based on the inhalation

of a small molecule that induced the endogenous production of a protein (an enzyme) was investigated.

In **Chapter 2**, three manufacturing methods were evaluated for their ability to produce a dry powder inhalation of cetorelix, a hydrophilic peptide that consists of 10 amino acids. Cetorelix was milled, spray dried and spray-freeze dried. The fragile structure of the spray-freeze dried powder made it unsuitable for processing to an adhesive mixture. The three methods resulted in particles with different morphologies, although all were in the appropriate size range for inhalation. The aerosolization behavior of the mixtures with milled and spray dried cetorelix was tested in two commercially available devices: a capsule-based inhaler and the Novolizer. The capsule-based inhaler uses the rotation and vibration of the capsule for deagglomeration, while the Novolizer utilizes a cyclone-based principle that uses impact forces for deagglomeration. When the adhesive mixtures of milled and spray dried cetorelix were dispersed with the capsule based inhaler and the Novolizer, both powder mixtures had appeared to have a higher fraction of cetorelix on the third and fourth stages of the cascade impactor when dispersed with the Novolizer. The morphology of the spray dried powder turned out to be better suitable for aerosolisation than that of the milled material in both inhalers. This investigation showed that the adequate development of inhalation systems requires a dual approach in which the specific combination of both the formation and device should be optimized.

In **Chapters 3 and 4** the hydrophobic peptide Cyclosporine A was investigated. Cyclosporine A is a cyclic undecapeptide, consisting of 11 amino acids. The inhalation of this drug could be used to prevent rejection of lung transplants. The utility of solid dispersion technology to manufacture a Cyclosporine A powder for inhalation was investigated. The solid dispersions were produced from a co-solvent system of water and *Tert*-butyl alcohol (TBA) using inulin with a degree of polymerization of 23 as carrier. After spray-freeze drying, a homogeneous powder Cyclosporine A and inulin with a high dissolution rate is produced. Fourier transform infrared spectroscopy (FTIR) was used to evaluate how Cyclosporine A was incorporated in the matrix. It was demonstrated that at increasing drug loads, more Cyclosporine A was present in a hydrophobic environment, indicating that more Cyclosporine molecules

were in close contact with each other, and possibly formed small clusters of even particles within the matrix. The formation of clusters might explain why powders with higher drug loads dissolved slower.

Spray-freeze drying results in low density particles with a very high specific surface area of more than 100 m²/gr. In spite of their large geometric size, the particles had still an aerodynamic size within the desired range of 1 to 5 µm, which is explained by the high porosity of the particles. The aerodynamic particle size was appropriate for inhalation, with a mass median aerodynamic diameter of 3.39 µm. The solid dispersion with 50% w/w CsA had the appropriate quality attributes for inhalation; a high dissolution rate and excellent aerodynamic behaviour.

Chapter 4 describes the *in vivo* properties of the Cyclosporine A powder for inhalation in rats. **Chapter 4A** focuses on pharmacokinetics and efficacy in preventing lung rejection of the Cyclosporine A powder for inhalation (iCsA) in a rat model. The pharmacokinetics of 10 mg/kg iCsA and Neoral, the oral commercial product containing Cyclosporine A, were studied. Although iCsA and Neoral resulted in comparable systemic exposure as determined by the area under the curve of the plasma concentration versus time curve and the same kidney exposure, iCsA had 109-fold higher lung tissue levels than Neoral.

In the proof-of-concept study, the rat model demonstrated acute rejection within 8 days as a result of the incompatibility of the donor and recipient. iCsA was already effective at a lower dose than Neoral. However, in the used iCsA dose (5 mg/kg) local reactions in the lungs of healthy, non-transplanted, rats were observed. Therefore, in **Chapter 4B** the effects of lower doses were studied and compared with the effects to placebo. At a dose of 0.8 mg/kg, iCsA seemed to cause no different effect as a placebo.

Although in Chapter 4A we demonstrated that doses lower than 5.0 mg/kg would not prevent rejection in the animal, the 0.8 mg/kg dose is relevant to humans, since it was described before that in man lower inhaled doses were already effective. We anticipate to administer a dose of 6.25 mg CsA twice daily, which would correspond to approximately 0.1 mg/kg for a 60-kg person. Even at such a dose, the local lung tissue levels of iCsA would increase substantially, while the contribution to systemic levels would be relatively small which reduces the risk of associated

nephrotoxicity. Hence, the efficacy of the treatment could possibly be improved in patients that otherwise have no therapeutic options left.

Chapter 5 describes a study into the possibilities of formulating a large protein, recombinant human deoxyribonuclease I (rhDNase) as a powder for inhalation, whereas **Chapter 6** provides a pharmacoeconomic analysis of such a product.

To formulate the protein we evaluated different excipients, processes and process conditions. In addition, the efficacy of the most suitable formulation was demonstrated with an *ex vivo* model. The excipient selection was performed by freeze drying deoxyribonuclease I in the presence of sucrose, trehalose and two types of inulin. Sucrose was found to be unsuitable as stabilizer, whereas trehalose appeared to be the best stabilizer. Furthermore, spray drying and spray-freeze drying were compared as potential manufacturing processes under different process conditions. Spray drying was the best suited process, as particles with a mass median aerodynamic diameter of 2.3 μm could be produced. Of the tested compositions only the inulin (DP 23) formulation could be stored at ambient conditions. The inhalation characteristics of this formulation were also good, with a fine particle fraction (< 5 μm) of approximately 40%. Therefore, we evaluated the visco-elastic properties of sputum from CF patients before and after applying the rhDNase/inulin DP23 formulation. The rhDNase powder for inhalation significantly decreased both elasticity and viscosity. We concluded that even larger proteins can be formulated as a powder for inhalation. Careful screening of excipients and processes may lead to the best possible combination, resulting in an inhalation powder with the best possible properties in terms of activity, storage stability and aerodynamic particle size.

Chapter 6 describes the pharmacoeconomic aspects of nebulization versus dry powder inhalation of rhDNase, a key product in the management of cystic fibrosis. The potential advantages of dry powder inhalation are *i*) improved compliance to therapy, *ii*) improved deep lung deposition, *iii*) no need for electricity of pressurized air, *iv*) reduced administration time and *v*) no need to cool the rhDNase solution. These benefits may among others lead to less exacerbations and less hospitalizations. The costs of rhDNase nebulization were estimated at €19.110 for a Life Year Gained (LYG), while the cost for a LYG of dry

powder inhalation was estimated at €12.100, which is 40% less than nebulization. This demonstrates that it may be useful to investigate already in an early stage of development what the pharmacoeconomic impact of certain formulation decisions may be.

In **Chapter 7** a highly innovative biopharmaceutical type of invention was explored. The small organic molecule, hemin was pulmonary administered in order to induce the endogenous production of a protein heme oxygenase I (HO-1). Such a approach would omit the difficult formulation of a protein for inhalation. HO-1 is a protein of interest because this enzyme is decreased in COPD patients compared to healthy subjects.

In the formulation process an alkaline solution of the poorly soluble hemin was neutralized immediately before spray drying, to yield a product containing hemin and sodium chloride. A new administration device for administering inhalation powders to small laboratory animals, using the natural breathing pattern, was also developed. It was demonstrated that HO-1 could be induced in a dose-dependent manner with inhaled hemin. This study showed that also with small organic molecules a biopharmaceutical intervention may be possible. Furthermore a novel method to allow preclinical testing of inhalation powders was introduced. This method is unique as it uses the inspiratory air flow of the animals, instead of “forcing” the powder into the lungs by insufflation. This opens the possibility to study the deposition characteristics of different powders in the preclinical setting.

In conclusion, the selected therapeutically active compounds could all be formulated as a powder for inhalation. Depending on the properties of the molecule, tailor-made formulation strategies could be employed to guarantee the structural integrity during drying and subsequent reconstitution. In some cases the pulmonary administration of a small molecule that induces the endogenous production of the therapeutic protein may be a good alternative, since it simplifies the formulation and less critical parameters need to be taken care of. As powders for inhalation rely on their aerodynamic properties for deposition and efficacy, there is a need to test the powders on these properties in a non-invasive manner in laboratory animals. In this thesis we used the insufflation and nose-only exposure approach. Insufflation is more suited for accurate dose-effect studies, while nose-only exposure can be used to

deliver a proof-of-concept, in which dose precision is less critical. In such a system, various formulation attributes (size, density) may be tested to find the best inhalable powder.