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Protein stability in pulmonary drug delivery via nebulization☆

Sebastian P. Hertel, Gerhard Winter, Wolfgang Friess *

Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig Maximilians-Universitaet Muenchen, Butenandtstr. 5, 81377 Munich, Germany

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ABSTRACT

Protein inhalation is a delivery route which offers high potential for direct local lung application of proteins. Liquid formulations are usually available in early stages of biopharmaceutical development and nebulizers are the device of choice for atomization avoiding additional process steps like drying and enabling fast progression to clinical trials. While some proteins were proven to remain stable throughout aerosolization e.g. DNase, many biopharmaceuticals are more susceptible towards the stresses encountered during nebulization. The main reason for protein instability is unfolding and aggregation at the air–liquid interface, a problem which is of particular challenge in the case of ultrasound and jet nebulizers due to recirculation of much of the generated droplets. Surfactants are an important formulation component to protect the sensitive biomolecules. A second important challenge is warming of ultrasound and vibrating mesh devices, which can be overcome by overfilling, precooled solutions or cooling of the reservoir. Ultimately, formulation development has to go hand in hand with device evaluation.

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1. Introduction

The rationale for pulmonary delivery of biopharmaceuticals can be ascribed to two different aims. On the one hand, systemic delivery through the lungs has been suggested to be a very promising non-invasive alternative to intravenous delivery of biopharmaceuticals featuring the unique combination of a highly disperse dosage form and a huge absorptive area directly interfacing with the blood circulation system [11,12]. Additionally the level of metabolizing enzymes is reduced compared to the GI tract [13] and absorbed molecules do not undergo a first pass effect [14]. Accordingly, bioavailabilities for proteins were reported to be the highest for any non-invasive route [15]. While insulin has been a driving force for research in the field, the withdrawal of Exubera® has also demonstrated some of the difficulties accompanying this approach [16–18].

On the other hand motivation for biopharmaceutical delivery to the lungs is the topical treatment of respiratory diseases. Direct access to the site of action allows for high local API concentrations while minimizing systemic exposure to the drug [10,19,20], making therapies more effective and safe. In 1996 Pulmozyme® (Dornase alfa, DNAse) became available for the treatment of cystic fibrosis as the first inhaled

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* Corresponding author.
E-mail address: wolfgang.friess@cup.uni-muenchen.de (W. Friess).

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biopharmaceutical. Unlike Exubera® it is also a market success with annual sales topping $600 million in 2011 and 2012 [21]. Local pulmonary delivery is an interesting opportunity for the treatment of respiratory diseases like CF, COPD, asthma or pulmonary fibrosis [22]. Various peptides and proteins are under development for the treatment of lung malignancies [23], lung transplant rejection [24], α1-antitrypsin deficiency (genetic emphysema) [25–28] or for pulmonary vaccination. An inhalative measles vaccination was reported to be superior to a parenteral vaccination in 4 million children [29].

Despite the advantageous biological situation and the successful example of Pulmozyme®, no other inhaled biopharmaceutical is marketed to date. A selection of biopharmaceuticals in development for inhalation is listed in Table 1. Many of the challenges of successful inhalation of a biopharmaceutical apply for both, local and systemic deliveries [30] although local treatment may seem an easier target [16].

A major obstacle is the achievement of sufficient and reproducible pulmonary API deposition, which is complicated by a highly branched lung geometry, different clearance mechanisms that prevent prolonged residence times and the great yet uncontrolled impact of the patient's breathing maneuver on aerosols deposition and distribution. At the same time aerosol generation must not corrupt the fragile stability of biopharmaceuticals to prevent a loss of biologic activity or unwanted toxic or immunogenic side effects. As demonstrated by the Exubera® failure, user-friendly and convenient operation of delivery devices is another requirement to gain market acceptance [16–18].

For aerosols to deposit into the lungs the branched geometry of the airways, mucocilliary clearance and phagocytosis by alveolar macrophages [80,81], that evolved to prevent the deposition of foreign material into the lungs, need to be overcome. This is achieved by the generation of an aerosol of sufficiently small particles by means of an inhaler device. The probability of aerosol deposition in the lungs is determined by the diameter (d) of the generated aerosol particles and the breathing maneuver performed by the patient [82] (v = speed — respiratory flow rate; t = time ~ breath holding time), which both influence the forces relevant for aerosol deposition mechanics [10,30,83,84]:

$$\text{Inertial impaction} \sim d^2 * v$$

$$\text{Gravitational sedimentation} \sim d^2 * t$$

$$\text{Diffusion by Brownian motion} \sim t / d.$$

Since breathing patterns differ substantially among individuals but are beyond control of common inhalers, pulmonary deposition of an

Table 1
Examples of proteins for inhalation*, in parts taken from [31,32].

Peptide/protein	Disease state	Device	Reference; clinical trial number
Dornase alfa (DNase)	Cystic fibrosis (CF)	Approved for nebulization; AERx	Genenzyme NCT01712334; [33–37] [38]
Recombinant alpha-1-antitrypsin (rAAT), alpha-1-proteinase inhibitor	Alpha-1-antitrypsin deficiency (AATD), CF	Nebulization	NCT00486837, Talecris, Phase III; [25], NCT01217671, Kamada, Phase III [39,40]
IgG1	Lung cancer	Nebulization DPI	[23,41] [42–44]
BIO-11006	COPD	Nebulization	NCT00648245; [45]; BioMarck, Phase II
IL-4/IL-13 antagonist (Pitrakinra)	Asthma	DPI	NCT00801853; [46]; [43], Aerovance; Phase II
rh-IL-4 receptor	Asthma	AERx	[47]
Bikunin (Aerolytic®)	CF, COPD	Nebulization	Aerovance; Phase II
Secretory leukoprotease inhibitor (SLPI)	Emphysema/CF	Nebulization	[48]
		DPI	[49]
Interferon-α	Tuberculosis, lung metastases	Nebulizer	[50,51]
		AERx	[52]
Interferon-β	Asthma	i-neb AAD	NCT01126177, Synairgen, phase II
	Respiratory viruses		
	Multiple sclerosis		
	Lung cancer		
Interferon-γ	IPF; tuberculosis; lung cancer Cancer/ pneumocystis carinii	i-neb AAD	[53]
Interferon-ω	Viral infections	Respimat®	[54]
Interleukin-2	Cancer	Jet nebulizer	[55,56]
Anti-IgE mAb (Omalizumab®)	Asthma	Jet nebulizer	[57]
		DPI	[58]
Catalase	Oxidative stress		[59]
Liposomal rh–Cu/Zn–superoxide dismutase	Acute lung injury	eFlow	[60]
Mn-Superoxide dismutase	Anti-inflammatory	Respimat®	[54]
Calcitonin	Osteoporosis	DPI	[61]
Parathyroid hormone	Osteoporosis	i.t.	[62]
Human growth hormone	Growth deficiency	DPI	[63]
Insulin	Diabetes	DPI	[64], Nektar/Pfizer withdrawn
Exubera		DPI	[65], NCT01451398
Afrezza		nebulizer	Mannkind, approved by FDA
Adagio™			Dance Biopharm Inc. EudraCT: 2012-002071-34
Insulin-like growth factor-I	Diabetes	Nebulizer	[66]
GLP-1	Diabetes	i.t.	[67]
rhG-CSF	Neutropenia	Nebulizer	[68]
GM-CSF	Pulmonary alveolar proteinosis; lung metastases	AKITA	[69]
		Jet nebulizer	[70–73]
Epo-Fc	Anemia	Nebulizer	[74,75]
IFN-α-Fc	Lung cancer, tuberculosis	Nebulizer	[76]
IFN-beta–Fc	Lung cancer	Nebulizer	[77]
	Multiple sclerosis		
FSH-Fc	Infertility treatment	Nebulizer	[78]
sFc-γ RIIb	Auto immune diseases	Nebulizer	[79]