International Journal of Pharmaceutics 532 (2017) 537–546

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Research Paper

A comprehensive screening platform for aerosolizable protein formulations for intranasal and pulmonary drug delivery

Martina Röhm[a,b], Stefan Carle[a,b], Frank Maigler[a,c], Johannes Flamm[a], Viktoria Kramer[a], Chrystelle Mavoungou[a], Otmar Schmid[d,e], Katharina Schindowski[a,*]

[a] Institute of Applied Biotechnology, University of Applied Sciences Biberach, Hubertus-Liebrecht-Strasse 35, 88400 Biberach, Germany
[b] University of Ulm, Faculty of Medicine, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[c] University of Applied Sciences Sigmaringen, Faculty of Life Sciences, Anton-Günther-Strasse 51, 72488 Sigmaringen, Germany
[d] Institute of Lung Biology and Disease, Helmholtz Zentrum München, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany
[e] Comprehensive Pneumology Center, Member of the German Center for Lung Research (DZL), Max-Lebsche-Platz 31, 81377 Munich, Germany

ARTICLE INFO

Keywords:
Antibodies
Scaffolds
Aggregation
Stability
Aerosolization
Nebulizer
Airways
Air-liquid interface
Permeation

ABSTRACT

Aerosolized administration of biopharmaceuticals to the airways is a promising route for nasal and pulmonary drug delivery, but − in contrast to small molecules − little is known about the effects of aerosolization on safety and efficacy of biopharmaceuticals. Proteins are sensitive against aerosolization-associated shear stress. Tailored formulations can shield proteins and enhance permeation, but formulation development requires extensive screening approaches. Thus, the aim of this study was to develop a cell-based *in vitro* technology platform that includes screening of protein quality after aerosolization and transepithelial permeation. For efficient screening, a previously published aerosolization-surrogate assay was used in a design of experiments approach to screen suitable formulations for an IgG and its antigen-binding fragment (Fab) as exemplary biopharmaceuticals. Efficient, dose-controlled aerosol-cell delivery was performed with the ALICE-CLOUD system containing RPMI 2650 epithelial cells at the air-liquid interface. We could demonstrate that our technology platform allows for rapid and efficient screening of formulations consisting of different excipients (here: arginine, cyclodextrin, polysorbate, sorbitol, and trehalose) to minimize aerosolization-induced protein aggregation and maximize permeation through an *in vitro* epithelial cell barrier. Formulations reduced aggregation of native Fab and IgG relative to vehicle up to 50% and enhanced transepithelial permeation rate up to 2.8-fold.

1. Introduction

Drug delivery to the airways using intranasal or inhalative aerosols represents a comfortable and non- to minimally-invasive way of self-administration. Although these administration routes are well established for small molecules, very little is known about the safe and effective administration of biopharmaceuticals in the airways. Most experience with aerosolized biopharmaceuticals has been gained for the delivery of desoxyribonuclease to the lung for the treatment of cystic fibrosis (Pressler, 2008), cytokines or cytokine agonists for the therapy of asthma (Thipphawong, 2006), but also with inhaled insulin (Siekmeier and Scheuch, 2008). Intranasal delivery with nasal sprays of the peptides desmopressin and calcitonin is well established for over 20 years (Ozsoy et al., 2009). As intransal delivery is also suitable to target the CNS, numerous studies delivered the peptide oxytocin (1 kDa) to treat social disorders (Hurlemann et al., 2010) and likewise the small protein insulin (5.8 kDa) that is able to improve the outcome of some cognitive tasks (Craft et al., 2012; Stützle et al., 2015). However, for larger and more complex proteins such as antibodies only few data are available demonstrating their feasibility as aerosols (Dellamary et al., 2004; Patton and Platz, 1992; Schüle et al., 2008).

Depending on aerosol size and inhalation manoeuvre, the aerosolization system allows targeted aerosol deposition in the nasal cavity (Engelhardt et al., 2016), nasal sinuses (Moller et al., 2014) and

Abbreviations: ALI, air-liquid interface; ALICE-CLOUD, Air Liquid Interface Cell Exposure system for aerosolized delivery of liquid drugs to cells cultured at the ALI; CNS, central nervous system; DoE, design of experiment; ELISA, enzyme linked immunosorbent assay; Fab, antigen binding fragment; FBS, fetal bovine serum; Fc, crystalizable fragment; FcRn, neonatal Fc receptor; FITC, fluorescein isothiocyanate; HBC, (2-Hydroxypropyl)-β-cyclodextrin; HPLC, high performance liquid chromatography; IgG, immunoglobulin G; KRB, Krebs Ringer buffer; mAb, monoclonal antibody; m_M, molecular mass; P_{app}, permeability coefficient; PS20, polysorbate 20/Tween® 20; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; SEC, size exclusion chromatography; SE-HPLC, size exclusion high performance liquid chromatography; SEM, standard error of the mean; TBS, Tris-buffered saline; TEER, transepithelial electrical resistance

* Corresponding author at: Institute of Applied Biotechnology, Biberach University of Applied Sciences, Karlstrasse 11, 88400 Biberach/Riss, Germany.
E-mail address: zimmermann@hochschule-bc.de (K. Schindowski).

http://dx.doi.org/10.1016/j.ijpharm.2017.09.027
Received 3 August 2017; Received in revised form 11 September 2017; Accepted 12 September 2017
Available online 14 September 2017

pulmonary respiratory tract (Coates, 2008). However, generation of micron sized aerosol droplets can have tremendous effects on proteins: complex proteins like monoclonal antibodies (mAbs) are very susceptible towards shear and mechanical stress experienced during aerosol formation, which can result in aggregation and unfolding of proteins (Maa and Hsu, 1997; Respaud et al., 2014). For instance, immunoglobulins have hydrophobic domains that can be adsorbed and enriched at the surface of liquid droplets where they interact with gas phase components. As a consequence, proteins are known to unfold, aggregate, degrade and denature (Bosquillon et al., 2004; Couston et al., 2012; Yu et al., 2006) resulting in decreased biological activity and immunological side effects (Rombach-Riegraf et al., 2014). The extent of aggregation is extremely dependent on protein structure, molecular mass, structural motifs, charge and hydrophobicity (Chiti, 2004).

Nebulizers are convenient devices for aerosolization of liquid drugs as they provide a moderate continuous flow rate and a constant aerosol size distribution in the optimal range for pulmonary or nasal delivery (Brun et al., 2000). Choosing a nebulizer exerting low shear forces during aerosol generation can reduce aggregation and loss of bioactivity (Andrew and Warren, 2015; Hertel et al., 2015). Likewise, it is well known that protein stability during aerosolization can be further improved by a suitable drug formulation (Shoyele and Slowey, 2006). Even though mAbs share the majority of the sequence in their constant regions, each mAb is unique and needs a tailored formulation (Frokjaer and Otzen, 2005; Wang, 1999; Wang et al., 2007). Interestingly, formulations can additionally have a positive effect on protein permeation through the mucosa and increase thereby bioavailability (Aungst, 2012; Patton and Byron, 2007; van der Lubben et al., 2001). Optimization of formulations consisting of various excipients typically requires systematic testing of a large number and combination of excipients. As nebulization and subsequent sample collection for each of these mixtures is very time consuming, an alternative surrogate method with high throughput capability is desirable. Recently, Hertel et al. (Hertel et al., 2014a) have shown that agitation of protein solutions can be used as surrogate for the stress caused by energy input during nebulization.

In a previous study, we have investigated the impact of aerodynamic particle diameter and flow rate on intranasal deposition using a vibrating mesh system (Engelhardt et al., 2016). The purpose of the present study was to develop a comprehensive and fast screening platform for protein aerosols assessing 1) the effect of excipients and formulations on protein stability during aerosolization and 2) the permeation of formulated, aerosolized proteins through an epithelial airway cellular model. For this study, an IgG and its antigen-binding fragment (Fab) were used. The number of required experiments for selection of an optimized formulation consisting of five different excipients is kept at a manageable level by using a previously published aerosolization-surrogate screening method (agitation instead of nebulization) combined with the statistical design of experiments (DoE) tool in a high throughput approach (Hertel et al., 2014a). Subsequently, transepithelial permeation of the best suited drug formulation was determined after aerosolized application onto nasal epithelial cells cultured under physiologic conditions at the air-liquid interface (ALI) using the ALICE-CLOUD aerosol-cell exposure system.

2. Material/Methods

2.1. Proteins

The mAb HIRMab 83-14 (designated here as IgG) is an agonistic antibody that binds to and activates the human insulin receptor (Krook et al., 1996; McKern et al., 2006). In ongoing studies, we use this IgG and its Fab as a powerful tool to compare intranasal bioavailability and efficacy of intranasal immunoglubulins with intranasal insulin, which is well described for intranasal delivery (Born et al., 2002; Craft et al., 2013). IgG and its Fab were produced and analyzed as recently described (Röhm et al., 2016). The hybridoma cell line producing this antibody was kindly donated by Ken Siddle (Cambridge University, UK). The antigen of IgG was absent in all studies to avoid interfering effects of antigen binding (data not shown). If not stated otherwise, samples were used at equimolar concentrations of 30 μM (1.4 mg/mL for Fab; 4 mg/mL for IgG).

2.2. Aerosol generator

A vibrating mesh nebulizer (Aeroneb Pro, Aerogen Inc., Galway, Ireland) was used in this study. The principles of operation of this device is depicted and described in Fig. 1A. Liquid passing through a vibrating membrane is dispersed into droplets with a mass median aerodynamic diameter of 2.5–6.0 μm. This clinically proven nebulizer is widely used due to its high liquid output rate of 0.3–0.8 mL/min and freely selectable air flow rate (air-less aerosol generation) (Longest et al., 2013). Here, 1 mL of the formulations was nebulized and aerosol droplets were either collected in a 15 mL Falcon tube and analyzed for monomer content or deposited onto epithelial cells for permeability measurements as described below.

2.3. SE-HPLC for the quantification of soluble and sub-visible aggregates

Size exclusion chromatography (SEC) was used for the detection and quantification of protein aggregates (Den Engelsman et al., 2011; Mahler et al., 2009). Non-nebulized, nebulized and agitated samples were analyzed using an UltiMate 3000 (Thermo Scientific, Langenselbold, Germany) high-performance liquid chromatography (HPLC) system equipped with a MAbPac™ SEC-1 size exclusion chromatography column (4 × 300 mm) combined with a precolumn MAbPac™ SEC-1 (5 μm, 300 Å, 4 × 50 mm; Thermo Scientific). The quantification of the monomer peak was normalized to a non-nebulized reference and displayed as percentage of monomer recovery [%].

2.4. Design of experiment (DoE)

To determine aggregate formation during nebulization in a small scale and rapid format, agitation was used as surrogate for nebulization as previously described (Hertel et al., 2014a). Briefly, 125 μL/well of formulated protein solution was transferred to a 96-well plate and constantly agitated with 900 rpm for 15 min at 30 °C in an orbital shaker (HLC, Ditabis, Pforzheim, Germany). For identification of an optimized stabilizing formulation, a central-composite-face centered design was used with five factors (excipients), which enables an estimation of linear and quadratic terms as well as first order interactions. (Eriksson, 2008). Three typically used concentration levels for each factor were investigated where the maximum, minimum and centre point concentrations are referred to as 1, −1, and 0, respectively. The investigated excipients were L-arginine, (2-Hydroxypropyl)-β-cyclodextrin (HBC), polysorbate 20 (PS20, Tween® 20), sorbitol, and trehalose (all purchased from Sigma Aldrich, Munich, Germany). The model contained 96 experimental runs (see Supplementary data). Six additional controls were implemented to better constrain the model by setting each factor to zero, while the other factors were held constant at their centre points and one control with all factors at their centre points. Monomer recovery [%] was determined by SE-HPLC as response factors and the data were fitted with the software MODDE 9 (MKS Data Analytics Solutions, Umeå, Sweden). The mathematical model was evaluated using partial-least square regression. Single, quadratic and interaction effects of the excipients were analyzed and non-significant coefficient factors (95% confidence interval includes zero) were removed (displayed in grey in the Supplementary table). The model was fitted until R^2 (model fit) and Q^2 (model prediction power) were optimal. The results were modelled with a polynomial equation (see Supplementary data).