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Dosing considerations for inhaled biologics

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ABSTRACT

The number of biologics in the therapeutic development pipeline is increasing including those delivered though inhalation (Morales, 2017; Fathe, 2016). Biologics comprise a broad variety of complex macromolecules with unique physicochemical characteristics. These distinctive characteristics control their pharmacological mechanisms of action, stability, and ultimately affect their processing, formulation, and delivery requirements. This review systematically covers crucial aspects of biologic powders formulations and dry powder inhalers which need to be taken into consideration to establish the drug loading and the payload to be delivered to reach the desired clinical dose.

1. Introduction

Establishing the proper dose for inhaled biologics in the lungs requires the integration of several factors Fig. 1): therapeutic dose requirements, formulation requirements (which ultimately leads to potency), and device delivery efficiency (Morales, 2017; Fathe, 2016). Biologics encompass a wide variety of complex macromolecules (proteins, peptides, vaccine, etc.) with diverse therapeutic applications and mechanisms of action, both at the local and systemic level. Stability is a critical aspect when developing biologics. Due to their more complex structure and intramolecular interactions beyond covalent bonds, macromolecules are generally more labile than small molecules and need protection during processing and for shelf life. This requires the addition of stabilizing excipients in the formulation, which will affect the drug loading of the final product. Finally, distribution and deposition of inhaled particles in the respiratory system ultimately determines drug concentration at the pulmonary epithelium. Therefore, particle engineering to achieve desired aerosol characteristics and device delivery efficiency are critical aspects for the development of a successful inhaled biologic. All these considerations, reported in more detail in this review, need to be evaluated to obtain a prototype formulation and device for preclinical tests from which the first in human dose will be established.

2. Biology

2.1. Inhaled systemically-acting biologics

Depending on the therapeutic indication, biologics may need to reach the systemic level to elicit their effect and this is ultimately governed by their bioavailability. In order to reach the systemic circulation from the lungs, a macromolecule must not only deposit in the target absorptive site of the airways, but also dissolve in the pulmonary surface lining fluids, and overcome the pulmonary epithelia barrier through the process of absorption (Ehrhardt, 2017). The absorptive surface area of the peripheral airways is significantly larger than that of the conducting airways and most biologics for systemic administration are thought to be best targeted to this region. Particle deposition in the respiratory track is governed by the aerodynamic diameter (D_a) of the particles, which depends on their physical diameter, density, and shape. Optimization of D_a through particle engineering will be discussed in more detail in the next section. In terms of particles dissolution, the remarkable absorptive surface at the air interface is covered by an extremely small amount of lining fluid (estimated to be 10–20 mL in healthy adults (Patton et al., 1998) allowing for an inhaled aerosol to be deposited in high concentrations in close proximity to lung vasculature. Pulmonary dissolution depends on many factors including the type of macromolecule and its physicochemical properties (e.g. lipophilicity/hydrophilicity), solubility in the airway lining fluids, and the powder formulation. In addition, compositions and volumes of the lining fluids might differ in different parts of lungs as well as clearance and protein degradation. Mucociliary clearance in the conducting airways is faster

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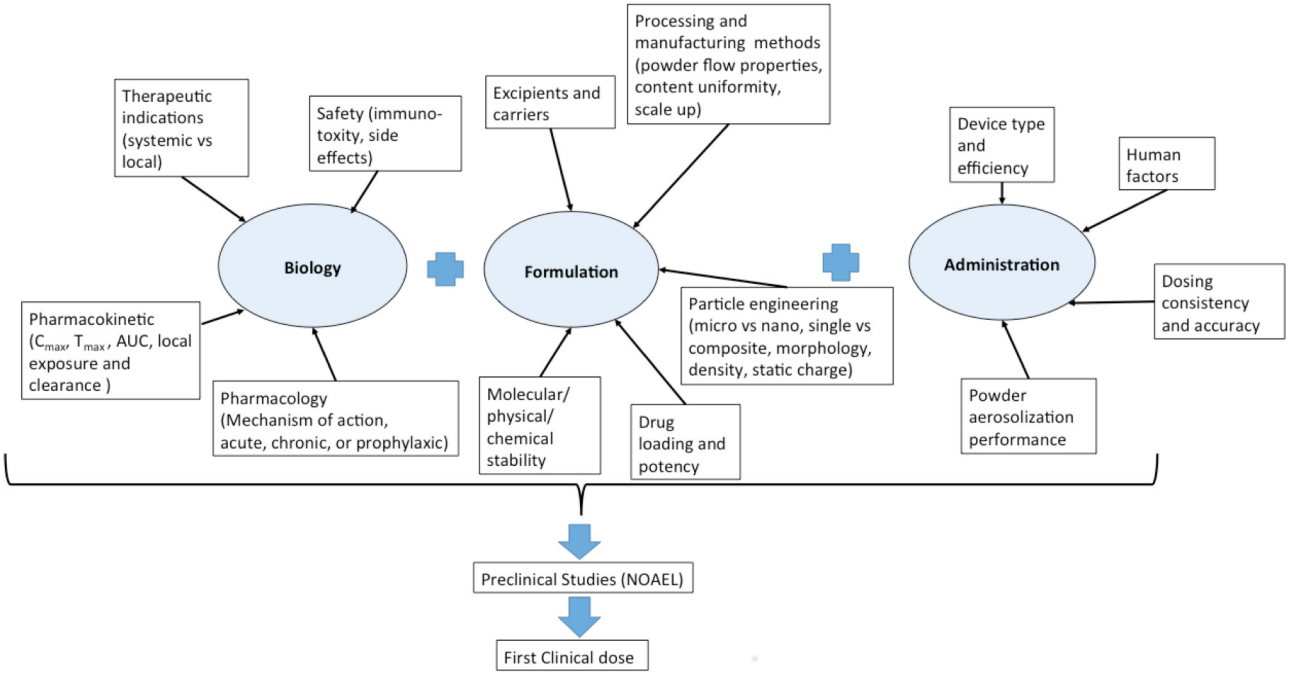

Fig. 1. Considerations for determining the dose of inhaled biologics.

Table 1
Cell culture models, table adapted from reference (Sakagami, 2006).

Epithelial cells	Human	Animal
Primary	Tracheobronchial Bronchial Alveoli (ATII)	Trachea Alveoli
Continuous (cancer derived)	Calu-1, Aclu-3, Calu-6 H441, HBE1 A427, A549	LA-4
Continuous (transformed from normal lung)	9HTE16o- 16HBE140- 1HAEo BEAS-2B CF/T43 AK-D	 L2 R3/1 MLE-7,-12,-15

compared with the slow macrophage clearance in the alveolar ducts (Ehrhardt, 2017) though phagocytosis itself can be rapid. In terms of degradation, lining fluid contains protease inhibitors that protect proteins from degradation (Patton et al., 1998; Patton, 1996). The release of protease into the lung lining fluid by immune cells usually occurs only during infection or chronic inflammation. However, exogenous macromolecules can still be degraded by macrophages. Typical inhaled particles have an aerodynamic diameter between 0.5 and 5 μm, however the average thickness of the alveolar lining fluid is 0.1 μm. Thus, only a limited amount of fluid is available for the dissolution of dry protein particles. Any aggregates, denaturated, or undissolved protein will be opsonized and targeted for macrophages uptake and degradation thereby reducing treatment efficacy.

Once the therapeutic powder is solubilized, it can be absorbed through the lungs via several different mechanisms generally categorized as either transcellular trafficking or paracellular transport. Transcellular trafficking, also called transcytosis, incorporates pinocytosis, caveolae-mediated transport, receptor mediated endocytosis, and phagocytosis (Patton, 1996). Paracellular, or the transport between cells, relies on tight junctions, endothelial junctions, or epithelial junctions (Patton, 1996). The mechanism of transport can have a significant impact on the pharmacokinetic characteristics of the treatment. For instance, if the molecules are transported through active mechanisms (Kim and Malik, 2003), the pattern of expression levels of the molecules' receptor in the lungs is important for understanding the kinetic of absorption and for optimizing drug delivery. As an example, the FcRn receptor's expression has been shown to be more abundant in the bronchial airways than the alveoli (Spiekermann, 2002). Thus its substrate, immunoglobulin G (IgG), can reach systemic circulation via transcytosis in the bronchial airways, despite less favorable barrier features for absorption including thicker epithelium and smaller total surface area (Sakagami, 2006).

A variety of *in vitro* cell models have been developed to increase understanding of pulmonary absorption mechanisms. Epithelial cells of alveolar or bronchial origin, obtained either from patient material or from established cell lines can form cell monolayers with functional intercellular junctions when cultured *in vitro* on permeable supports either in liquid condition or at the air-interface interface. These *in vitro* models enables the systematically study of the trans-epithelial transport kinetics of test molecules (see Table 1). Immortalized and primary cell lines have been derived from either lung cancer epithelial cell lines (A549, Calu-3) or from normal human/animal lung cells transformed by viruses (16HBE14o- and BEAS-2B) (Sakagami, 2006; Horálková, 2009; Douglas and Kaighn, 1974; Ehrhardt et al., 2008; Bosquillon, 2017). Due to their continuous replicative capability in culture, immortalized cell lines may offer more reproducibility and ease of use for scientists. On the other hand, primary cultured cell models can be more representative of *in vivo* transport kinetics. In general, caution is required for *in vitro-in vivo* correlation since properties of the monolayer have been shown to differ with culture conditions, e.g. several lung epithelial cell models cultured under an air-interface display different behavior as compared to growth under submerged cultures (Ehrhardt, 2002). In addition, although the expression and functionality of numerous transporter proteins have been found in these monolayer models (such as IgG FcRn receptor, albumin-binding glycoprotein gp60, and efflux protein, P-gp), the kinetic contribution of such active absorption mechanisms in intact lungs has not been fully understood, and it is still unclear whether these receptors' expression pattern is comparable between *in vitro* models and *in vivo* conditions. Therefore, the relevance of using single cell barriers models to simulate complex kinetics of absorption is debatable, despite their increasing use in the