Insights on animal models to investigate inhalation therapy: relevance for biotherapeutics

Guillon A¹,²,³, Sécher T¹,², Dailey LA⁴, Vecellio L¹,⁵, De Monte M⁶, Si-Tahar M¹,², Diot P¹,²,⁷, Page CP⁸, Heuzé-Vourc’h N¹,²

1. INSERM, Centre d’Etude des Pathologies Respiratoires, U1100, F-37032 Tours, France
2. Université François Rabelais de Tours, F-37032 Tours, France
3. CHRU de Tours, Service de Réanimation Polyvalente, F-37000 Tours, France
4. Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06122 Halle (Saale), Germany
5. Aerodrug, Université François Rabelais - Faculté de Médecine, Tours, France
6. Plateforme Scientifique et Technique (PST) Animaleries, Université F. Rabelais, F-37000 Tours, France
7. CHRU de Tours, Service de Pneumologie, F-37000 Tours, France
8. Sackler Institute of Pulmonary Pharmacology and Institute of Pharmaceutical Science, King’s College London, 150 Stamford Street, London SE1 9NH, UK

Corresponding Author:
Dr Heuzé-Vourc’h Nathalie
INSERM, Centre d’Etude des Pathologies Respiratoires, U1100
Université François Rabelais de Tours
10 Boulevard Tonnellé
F-37032 Tours, France
nathalie.vourch@med.univ-tours.fr
Tel : (0033)247366237; fax : +33 2 47 36 60 46

Graphical Abstract
Abstract

Acute and chronic respiratory diseases account for major causes of illness and deaths worldwide. Recent developments of biotherapeutics opened a new era in the treatment and management of patients with respiratory diseases. When considering the delivery of therapeutics, the inhaled route offers great promises with a direct, non-invasive access to the diseased organ and has already proven efficient for several molecules. To assist in the future development of inhaled biotherapeutics, experimental models are crucial to assess lung deposition, pharmacokinetics, pharmacodynamics and safety. This review describes the animal models used in pulmonary research for aerosol drug delivery, highlighting their advantages and limitations for inhaled biologics. Overall, non-clinical species must be selected with relevant scientific arguments while taking into account their complexities and interspecies differences, to help in the development of inhaled medicines and ensure their successful transposition in the clinics.

Keywords: Animal models; Inhalation therapy; Biotherapeutics

1. Introduction

Over the last decades, the respiratory drug pipeline has broadened to include a large array of biotherapeutics which have ushered in a new chapter in the management and treatment of patients with respiratory diseases (Reichert, 2016). As an example, in the past two years two monoclonal antibodies (mepolizumab and reslizumab) received approval for severe asthma, providing further options for the treatment of severe asthma. When considering the administration of large drug molecules, like biotherapeutics, matching the delivery route to the relevant anatomical location is paramount (Desoubeaux et al., 2016). The delivery of drugs for treating respiratory diseases by inhalation has long been considered to allow a rapid onset of action, to ensure a high concentration of drugs in the vicinity of the relevant anatomical region while limiting systemic exposure and reducing the likelihood of side-effects. Inhalation is commonly used for small molecule drugs to improve their therapeutic index, but appears more complicated for biotherapeutics, with only one protein, Dornase alpha (Pulmozyme™), approved for the treatment of cystic fibrosis by inhalation (Dentice and Elkins, 2016), and few additional inhaled biotherapeutics in clinical evaluation (alpha anti-trypsin, an anti-RSV nanobody™ and two monoclonal antibody (mAb) fragments). Indeed, inhalation of biotherapeutics raises technical and formulation challenges (e.g. stability during aerosolization), pharmacological issues related to their fate and action after pulmonary deposition, and safety issues (Respaud et al., 2015). To improve the development of inhaled biotherapeutics and avoid failure to translate promising drugs from animal models to humans, it is crucial to use/develop more predictive preclinical models. For the development of inhaled drugs, local lung tolerance is one of the most critical aspects and requires the use of several animal species. On the other hand, assessing in vivo lung deposition, pulmonary pharmacokinetics (PK) and pharmacodynamics (PD) are also essential to understand the fate and specific effects of inhaled liquid or solid (powder) formulations. Given the variability of the deposition of inhaled particles in the airways, as well as their clearance and pharmacological effects, a large number of animal species have been used in preclinical studies. Rodents (rats and mice) are the most commonly used species, but larger mammals such as dogs and primates are often
required for toxicological evaluation. Although animals are necessary, they all display their own limitations as models of humans. Thus, when considering non-clinical studies of inhaled materials in experimental mammals it is imperative to take into consideration the anatomy of the airways, the characteristics of particle deposition and clearance, as well as methods of exposure (inhalation chambers, masks, etc...), and the practical design of the experimental protocol. Other factors are also important, such as biotherapeutic interaction with the molecular target and animal host system, ease of handling animal models, and technical constraints linked to animal housing and/or the availability of specific techniques. This review describes existing approaches to the use of experimental animals regarding aerosol drug deposition, PK/PD and toxicity, and discusses the limitations of these models with respect to the anatomical and physiological characteristics of the respiratory system.

2. Animal models and aerosol delivery

Studies dealing with modelling and/or comparison of deposition of inhaled particles by the most common laboratory species are based on a limited amount of data (Derelanko and Hollinger, 1995; McClellan and Henderson, 1989; Schlesinger, 1985). It is likely that total deposition of an inhaled aerosol might be similar in mammals with equal mass, despite distinct anatomical features, whereas there might be greater inter-species variations in terms of regional deposition (Phalen et al., 2008). Overall, anatomical characteristics of the lungs and respiratory parameters differ considerably among mammals and clearly matter when evaluating aerosol deposition and for translating results from animal models to humans.

2.1 Rationale for species selection

Many species are suitable for studies of aerosol deposition of drugs in the lungs, but a rigorous comparative analysis of the airway anatomy and physiology is mandatory in the selection of an animal model in order to extrapolate the results to humans. The structure of the respiratory apparatus changes significantly across species with regard to the anatomy of the nose, the overall anatomy of the lung, the tracheobronchial tree, and the lower lung. Interspecies comparisons of breathing parameters have been reviewed in more detail elsewhere (Cryan et al., 2007) and key differences in individual anatomical, biological and physiological parameters are discussed below.

2.1.1 Comparative anatomy of the lung, from the nose to the alveoli

Small laboratory rodents are unable to breathe through the mouth, breathing only through the nose, similar to humans during the neonatal period. In contrast, most other mammals preferentially breathe through the nose, although they are also able to breathe through the mouth, as do pigs in hyperventilation or non-human primate (NHP) with nasal obstruction. The nose in humans and other primates consists of several superimposed shelf-like structures (turbinates) projecting into the nasal cavities with a small olfactory region. The turbinates cause turbulence of the inhaled air. In contrast, the nasal anatomy of most other mammals is more complex, with double-scrolled turbinates and large olfactory regions (Schreider and Raabe, 1981). Anatomical comparison can be misleading because the nasal cavity is not uniformly ventilated. Only 10-15% of the inspired air-stream follows a dorsal pathway of the rat nose and flows over the complex ethmoturbinate. Thus, whereas the
The ethmoturbinate structure in the rat is quite different than in the human, little of the inspire air passes through this region (Salem and Katz, 2005). Magnetic resonance imaging (MRI) studies have shown that there are many similarities in the structure of the monkey and human nose suggesting that the rhesus monkey is a good human surrogate in aerosol deposition studies (Yeh et al., 1997). In contrast to humans, nose-breathing rodents exhibit a lower pulmonary deposition accompanied by a higher nasal and tracheobronchial deposition, when inhaling large particles (> several µm) (Schlesinger, 1985). When comparing pulmonary aerosol deposition of large particles, in conditions of nasal breathing between humans and other species, deposition is similar in humans and NHP, but greater in humans than in rats (Phalen et al., 2008). Interestingly, adapting the aerosol device to minimize large particles may reduce the nasal particle trapping, as demonstrated in guinea pigs (Wu et al., 2011).

Some of the main anatomical features of the respiratory tract of laboratory species are compared to those of humans in Figure 1. The size of the thoracic cavity varies according to the species, which consequently affects the size and volume of the lungs. The overall shape of the airway is of particular significance, as seen in the relatively dichotomous and symmetrical branching scheme of the tracheobronchial system of primates as compared to the long tapering monopodial airways of small rodents. These anatomical variations may introduce changes in direction of the airflow favoring aerosol impaction and thus alteration in deposition pattern (Warheit, 1989). Such particular impaction “hotspots” at bifurcations in the human tracheobronchial tree have been described in detail elsewhere (Balashazy et al., 2003). In species with monopodial branching patterns, and more particularly in the rat, the particles carried in the airflow continue into the major daughter airways with little change in velocity or direction, with deposition occurring largely by sedimentation (Warheit, 1989). Rodents also differ from humans and NHPs in that they have no respiratory bronchioles. In the rat, the terminal bronchioles, the smallest conductive part of the respiratory tract, are connected directly into the alveolar ducts (Phalen and Oldham, 1983; Tyler, 1983). Mammals are also distinguished by the number and size of the pulmonary acini. The size and number of alveoli and the lung area increase with the size of the animal (Derelanko and Hollinger, 1995). Lobulation of the lung is more or less pronounced among species. The left lung is generally divided into two lobes (e.g. humans, pigs, rabbits, guinea-pigs) or three (e.g. rhesus or cynomolgus monkeys) and the right lung into three. The left lung remains single-lobed in certain rodents such as the rat and the mouse, while the right lung is divided into four lobes.

2.1.2 Impact of specific physiological and biological characteristics on aerosol administration

In addition to inter-species anatomical differences, there are considerable inter-species physiological and biological differences in lungs that may impact relevance of animal studies to predict aerosol deposition in humans. First, there are significant differences in the lung volumes and breathing pattern across different species. As summarized in Figure 2, the tidal volume increases with body weight, but in contrast, the respiratory rate drastically decreases with an increase in body weight. Thus, extrapolation of results from lung deposition studies in mice to humans is challenging. Second, the rate of inhaled particle absorption is not homogenous across species. Absorption of lipid-insoluble drugs varies importantly according to species, whereas lipid-soluble drugs are equally absorbed into the bloodstream of various animal species and are poorly affected by clearance mechanisms (Phalen et al., 2008; Schanker et al., 1986). Moreover, non-clinical studies are often
carried out in animals at an early stage of life while the rates of lung absorption vary according to age (Schanker and Hemberger, 1983).

Complex mechanisms of non-absorptive clearance following lung deposition can also modify the bioavailability of drugs in the lung, such as mucociliary clearance, protease digestion and/or phagocytosis. Indeed, morphometric investigations in rats, dogs, baboons, and humans revealed variations in the distribution of alveolar macrophages (Crapo et al., 1983). Human lungs contained significantly greater number of alveolar macrophages which may have implications for non-absorptive clearance (Crapo et al., 1983). Inhaled particles are cleared from the tracheobronchial region by the ciliary action of the epithelial cells lining the airways, the so-called “mucociliary escalator”. The speed of tracheobronchial clearance is difficult to compare between species since the size and length of the tracheobronchial tree are highly heterogeneous. Furthermore, small rodents (mice, rabbit) lack bronchial submucosal glands (March et al., 2000). Finally, distribution of the pharmacological effectors/modulators may be distributed differently within the lungs in animal species and limit result translation to human. As an example, the neonatal Fc receptor (FcRn), known for its role in IgG homeostasis, is a critical factor in the biodistribution and PK of mAbs and is differently expressed across species (Roopenian and Akilesh, 2007). FcRn is expressed by numerous cell types such as epithelial cells, endothelial cells, and macrophage/monocytes, in which it prevents IgG degradation in the lysosomes and contributes to IgG recycling and transcytosis. While FcRn is expressed by the alveolar macrophages of all species, there are differences in the sites of FcRn expression in the respiratory epithelium. FcRn expression is restricted to the epithelial cells of the upper airways in primates, whereas it is also detected in the epithelium of the alveolar region in rodents.

2.2 Methods of aerosol administration

2.2.1 Non-invasive aerosol delivery in spontaneously breathing animals

Whole-body inhalation. Whole-body inhalation systems are particularly convenient for inhalation studies using large numbers of animals. Another advantage of whole-body inhalation systems is the ability to expose conscious animals without restraint, thereby limiting experimental artefacts. However, a large proportion of the aerosol is deposited on the animal’s fur, with low lung deposition (<10% of the administered dose) and confounding PK results due to intestinal absorption. For biotherapeutics such as mAbs, protein degradation in the stomach is probably associated with a low transfer of the intact drug into the bloodstream. Thus, one would assume that the gastrointestinal-absorption may poorly interfere with PK of inhaled biotherapeutic drugs. In addition, the size of inhaled particles cannot be controlled, and the exact quantity of drug administered to the lung is difficult to determine (Kawajiri et al., 2004; March et al., 2004; Pauluhn, 2006). Furthermore, this system is not recommended for expensive drugs, like biotherapeutics, because of important drug loss. Another confounding factor is coming from the fact that animals are in a horizontal position (standing on four legs) during aerosol delivery, which favors ventral lung deposition. For these reasons, whole-body inhalation systems are rather used to simulate airborne virus infection (Belser et al., 2015) or cigarette smoke exposure (Guillon et al., 2015b).

Nose-only inhalation. Nose-only inhalation systems are generally preferred to reduce absorption by non-respiratory routes. This system also affords measurement of respiratory parameters by plethysmography.
during exposure, thereby allowing variability in dosing due to differences in individual animal breathing patterns to be addressed (Pauluhn and Mohr, 2000). Nonetheless, a large amount of aerosol is still deposited in the nasal cavity, due to nasal breathing of experimental animals. This fraction may be transferred to the gastrointestinal compartment following mucociliary clearance where it can be absorbed into the bloodstream. This may impact the interpretation of the results of PK or toxicity studies. As previously mentioned gastrointestinal absorption may poorly interfere with PK of protein therapeutics. In contrast, biotherapeutic deposition in the nose and further direct adsorption may biased PK analysis. Indeed, recent data supports expression of FcRn in the nasal epithelium and its role in antibody/IgG transcytosis through the nasal epithelium (Heidl et al., 2016).

**Face-mask inhalation.** Large mammals can breathe spontaneously through a face mask connected to an aerosol generator (Supplementary figure 1) (Marchand et al., 2015). However, because the facial features of the animal differ from those of a human, a mask interface is necessary to connect human devices, such as pediatric aerosol generators, nebulizers or pMDIs with inhalation chambers. Although, a quantity of aerosol is lost in the device and the mask interface, a significant quantity of the aerosol is deposited in the respiratory tract. Interestingly, face-mask inhalation in macaque has been recently used for the preclinical development of a drug and device system envisioned for the delivery of a mAb into the deep lungs of adults. As expected, mAb deposition in the lungs of macaque was lower than the dose predicted by cascade impaction, because macaque have ventilation parameter comparable to infants (Respaud et al., 2016). Other limitations include the requirement of animal training prior to aerosol administration and the use of anesthetics that can affect breathing parameters (Iizuka et al., 2010). Despite the limited relevance of these methods to evaluate the relationship between the effectiveness/toxicity and the dose administered, and the difficulties to accurately predict lung deposition, these methods are used and validated for lung tolerance and regulatory toxicity studies. Like for other methods described above, gastrointestinal deposition is important by face-mask inhalation - probably consecutive to direct swallowing or licking and subsequent swallowing of the drugs deposited in the mouth and/or on the face – but may have a limited impact on protein therapeutic pharmacology (Respaud et al., 2016). For example, face-mask inhalation has been used during the preclinical development of ALX-0171, an anti-RSV nanobody™, to assess PK and anti-viral effects in a neonatal lamb RSV infection model (Larios Mora et al., 2015). It is also worth noting that these methods could be particularly useful to study lung tolerance following repeated drug exposure (Pauluhn, 2014).

### 2.2.2 Intra-tracheal aerosol delivery

**Intra-tracheal delivery in small rodents.** To bypass the oro-pharynx during administration and ensure a full dose delivery in the lung, intra-tracheal instillation has been widely described in anesthetized animals using either the Saffioti’s method (Saffioti et al., 1968) or alternatively following tracheotomy or orotracheal intubation. To overcome issues associated with intra-tracheal instillation, a large number of devices allowing the delivery of a spray/aerosol directly into the trachea of rodents have been developed (Chen et al., 2002; Hamacher et al., 2008; Rivera et al., 2005; Spoelstra et al., 2007; Todo et al., 2003). Intra-tracheal administration in rodents using spray/aerosol devices, such as the MicroSprayer® Aerosolizer and Dry Powder Insufflator™ (Penn-Century Inc., US), allows the rapid and reliable delivery of a controlled quantity of drug directly into the lungs (Supplementary figure 2) (F. Gagnadoux et al., 2005; Mercier et al., 2014). These devices
can be used with liquid or solid drugs (e.g. anti-cancer agents and anti-fungals) or excipients at single or repeated doses, to assess efficacy, tolerance and PK (Chandenier et al., 2009; Frédéric Gagnadoux et al., 2005; Gagnadoux et al., 2008; Maillet et al., 2011; Montharu et al., 2010). The Microsprayer™ device has been used with success to assess the efficacy of several mAbs in murine models (Guilleminault et al., 2014; Hervé et al., 2014; Maillet et al., 2011). It should be noted however that tolerability issues have been raised concerning intra-tracheal administration with these devices in small rodents (slight and transitory lung inflammation (Dubois et al., 2013; Montharu et al., 2010) and lung lesions (Guillon et al., 2012)). A nebulization catheter device (AeroProbe™, Trudell Medical International, Canada) was also commercialized to administer aerosols directly into the airways and to provide a complementary approach to existing methodologies for pulmonary drug delivery in small animals (Tronde et al., 2002). However, to the best of our knowledge, these devices are no longer commercially available. Aerosol delivery systems for tracheal intubated and mechanically ventilated mice have also been described (Robichaud et al., 2015). These nebulizers are integrated into the inspiratory branch of the ventilator; however, laboratory-related protocol variations greatly affect the efficiency of the aerosol generation process and avoid study comparisons (Robichaud et al., 2015).

In certain experimental scenarios (e.g. when a very mild anesthesia is necessary), alternative administration techniques, such as oropharyngeal aspiration or intranasal administration may be used. Oropharyngeal aspiration is limited to the administration of liquid solutions or suspensions to the lungs, as it involves applying a small droplet (typically 25 to 50 µL per mouse) at the top of the pharynx and allowing the droplet to be aspirated into the lungs during normal inhalation (Foster et al., 2001; Lakatos et al., 2006; Rao et al., 2003). Although, this dosing technique is primarily used in the generation of murine disease models (such as chemically induced asthma (De Vooght et al., 2009; Morgan et al., 2008) or bleomycin-induced lung fibrosis (Babin et al., 2012)), it was used with success to deliver biotherapeutics (Respaud et al., 2016). It was also demonstrated that oropharyngeal aspiration generated similar lung deposition profiles as MicroSprayer® Aerosolizer instillation, notwithstanding a quicker overall process and the requirement for milder anaesthetic regimes (Patel et al., 2016; Woods et al., 2015). However, this method may induce a bias in PK studies due to intestinal absorption. Overall, commercial intra-tracheal administration devices, unlike traditional aerosol administration devices (inhalation chambers), allow the dose of the product to be controlled, ensuring a high deposition rate (70 to 80% of delivered dose) and a relatively uniform distribution of the product in the lungs of larger rodents, such as rats. They also allow rapid and repeated administrations of the drug into the lungs, which is suited to evaluate the efficacy of inhaled biotherapeutics. Finally, one has to consider that intra-tracheal aerosol delivery in obligate nasal breathers is not exempted of drug deposition in the nasal cavity. Indeed, a part of the aerosol still floats and remains in upper airway after the aerosol delivery. When the animal exhaled through the nose, the aerosol could also be transported on the walls of nasopharynx (Yamamoto et al., 2017).

**Intra-tracheal delivery in large mammals.** Intra-tracheal administration in intubated large animals is particularly relevant to mimic aerosol delivery during invasive mechanical ventilation in humans. Primates can be intubated and mechanically ventilated using respiratory parameters mimicking “pediatric patients”. Pigs are commonly used as a model for aerosol administration to ventilated patients in intensive care units (Rouby et al., 2012). In these models, human devices can be used without any modifications and synchronized to inspiratory time if needed. Despite their tracheal bronchus and the difficulty of oro-tracheal intubation due to their long mouth cavity and curved larynx, the lung anatomy and physiology of pigs are close to that of humans, which
enable extrapolation of experimental results of aerosol deposition to humans (Supplementary figure 3). Presently, this model has mainly been used to evaluate nebulization of topically-acting small drugs (e.g. antibiotics) during artificial ventilation (Rouby et al., 2012). Indeed, pigs are becoming an increasingly important species for translational research and may be a relevant model in which to test the efficacy of anti-infectious biotherapeutics. A major advance was made in the field of respiratory medicine with the generation of the porcine cystic fibrosis model (Guillon et al., 2015a; Rogers et al., 2008). Sheep have also been used, but less frequently, as a model for aerosol delivery during mechanical ventilation. One of the best examples has been provided during the preclinical development of a 45kDa recombinant alpha antitrypsin (rAAT) that demonstrated the feasibility of using aerosolization to the pulmonary surface to administer rAAT in sheep (Hubbard et al., 1989). Direct intra-tracheal administration of liquid or powder aerosol is also possible in intubated large animals thanks to dedicated sprayers (Guilleminault et al., 2014). Dry powder aerosols can be administered using a Dry Powder Insufflator™ without any concerns for tolerance considerations associated with the device (Guillon et al., 2016); the volume of insufflated air generated by the device is considerably lower than the animal’s tidal volume and therefore does not cause lung lesions (Guillon et al., 2012). Dry powder inhaler devices have also been developed for use in beagle dogs via oro-tracheal intubation (Sellers et al., 2015).

2.3 Aerosol delivery methods in animals and transposition to human

It is important to keep in mind that inhalation therapy is atypical in its difficulty to find relevant animal models, compared to other routes of delivery. Pulmonary delivery is probably the most challenging route in animal models because drug delivery is highly dependent of the anatomy and respiratory physiology of the animal species. Drug deposition in animal is also affected by the aerosol-generating systems, which poorly mimic the aerosol distribution encountered in human, even when using devices intended for human. Consequently, (i) the gap between human and animal models anatomy and respiratory pattern, and (ii) the differences between devices engineered for experimental purpose and actual human devices, are two important limiting factors for successful translation to the clinic. It is clear that further refinement of current experimental models is necessary. The development of animal devices that would reproduce the distribution of the drugs in human, taking into account the respiratory parameters, the physiological properties and anatomy of the lungs of the animal, may be helpful for the translation of results into the clinics.

Despite those limitations, two animal models of aerosol delivery have a high translational potential for specific clinical applications and should be highlighted. First, aerosol delivery during invasive mechanical ventilation in large mammals, such as pigs, is particularly relevant to mimic aerosol delivery during invasive mechanical ventilation in humans. As the oropharynx is bypassed by the tracheal probe, the anatomic differences are not anymore issues. Moreover, ventilators settings could be identical between humans and pigs. In a recent systematic review assessing the aerosol delivery during invasive mechanical ventilation according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, 36 studies were finally included among 234 articles assessed for eligibility, and comprised 26 clinical studies and 10 experimental studies (Dugernier et al., 2017). Nine of these experimental studies were performed on pig models and one a dog model. So far, these models have never been used for the development of a biotherapeutic. The
second relevant model is the macaque (Macaca Fascicularis), in the weight range of a full-term newborn human, which enable to make accurate prediction of aerosol lung deposition in infant (Dubus et al., 2005; Réminiac et al., 2017). No modification of human facemask is necessary; infant are also nose breather not able to synchronize inspiratory time with drug delivery.

3. Methods to assess lung deposition after aerosol delivery

Distribution and deposition of the aerosol in the different regions of the lung depend on particle properties, but are also affected by the subject’s breathing pattern pathophysiological context. Hence, the dose of the delivered aerosol is likely to vary substantially in the lung and imaging provides a non-invasive way to measure in vivo the regional distribution of inhaled drugs.

Assessment of aerosol deposition in airways is mainly performed using imaging techniques which are summarized in Table 1. Among them, planar (2D) scintigraphy is the most relevant and standardized method, providing robust results (Newman et al., 2012). It relies on the use of either direct or indirect radiolabeling of the material to be measured using a gamma camera, and provides a 2D image of the distribution of radioactivity in the airways. Technetium-99m (99mTc) is the most common radioactive element used for labeling due to its suitable half-life regarding the duration of image acquisition and radio-protection. It can be also used in combination with diethylene triamine penta-acetic Acid (DTPA) to trace the unlabeled drug and avoids quick 99mTc diffusion into the systemic circulation (Guillon et al., 2015c).

Nebulizer delivery methods are well adapted to 99mTc-DTPA 2D scintigraphy because the active drug is directly accessible for labeling (Behr et al., 2009). Biotherapeutics can also be used as theranostic agents through the attachment of a radiolabel chelator to the biotherapeutic. For antibodies, the use of the short-lived 99mTc improves physical imaging because of high proton flux, high resolution and low dosimetry (Andersson et al., 2016). It is also particularly relevant for Fab fragments which exhibit accelerated clearance resulting in better target-background ratio (Wahl et al., 1983). Quality specifications of a labeled biopharmaceutical must be assessed and in particular the conservation of its biological properties (Eisenhut and Haberkorn, 2006). In addition, the characterization of peptide impurities following labeling or instability processes is mandatory as such by-products can generate toxic or unexpected pharmacological effects. Labeling is more challenging when complex devices or formulations are involved, such as pMDIs or DPIs, as this requires manufacture and filling of the radioactive formulation into the delivery device (Aug et al., 1991). The validation of aerosol drug radiolabeling and radiotracing must be done prior to the 2D gamma camera imaging. It must be demonstrated that the radiolabel or radiotracer does not affect the aerosol characteristics compared to the non-labeled system (Guillon et al., 2015c). Recommendations have been established for the validation of a radiolabeling/radiotracing method, utilizing similar criteria to those recommended by the EMA guidelines and the ISAM/IPAC-RS Workshop Report (European Medicines Agency Committee for Medicinal Products for Human Use, n.d.).

Multiple planar images at different positions around the body can be processed to generate a 3D-image of the location of a radiolabeled drug. This principle is at the basis of both positron emission tomography (PET) and single-photon-emission computed tomography (SPECT) imaging. Before PET became available, SPECT was the primary imaging modality, especially to assess mAb distribution. SPECT imaging relies on similar
radioelements as planar scintigraphy, including $^{99m}\text{Tc}$, $^{111}\text{In}$ or $^{131}\text{I}$. Both fragment and full-length mAb have been labeled and the rationale supporting the choice of the radioelement is based on the matching between its half-life and the plasma residence time of the mAb. For example, long-lived $^{111}\text{In}$ or $^{131}\text{I}$ have been used to label whole antibodies, while the short-lived $^{99m}\text{Tc}$ has been used to label antibody fragments and all have been successfully imaged in humans (Goldenberg et al., 1978; Machac et al., 2002; Nagengast et al., 2011; Yao et al., 2007). PET uses radioisotopes that emit a positively charged electron, which when once annihilated produces two gamma rays in exactly opposite directions allowing for a better resolution than SPECT. Among the PET radioisotopes, $^{89}\text{Zr}$ has been widely used in the clinic for labeling anti-tumor mAbs. Short-lived positrons like $^{18}\text{F}$ or $^{76}\text{Br}$ are mainly used for the imaging of antibody fragments and small antibody-like molecules (Lamberts et al., 2015). Of note, scintigraphy acquisition required careful adjustments and corrections that are not detailed here. These correcting factors may vary depending on the anatomical features of the animal species (Frédéric Gagnadoux et al., 2005; Gagnadoux et al., 2006; Guillon et al., 2014). In many cases, anatomical images can be added to deposition images using X-ray computed tomography (CT) or magnetic resonance imaging (MRI) to provide a better spatial distribution of the aerosol and evaluation of aerosol treatments (Conway et al., 2013). These methods provide considerable amounts of information in terms of aerosol distribution in the lung and they are mainly used for basic scientific research. Nevertheless, they are more complex methods than 2D scintigraphy using a gamma camera and the algorithms for calculating aerosol deposition are less well established. MRI, on its own, has also been considered in assessing lung aerosol deposition and has the advantage of measurement without radioactive agent exposure (Thompson and Finlay, 2012). However, the oxygen in the air that filled lungs complicates MRI, thus making necessary helium inhalation to enhance contrast in the lungs. For a long time, the signal intensity of the contrast agent in the lung and the lack of a standardized method of calculation limited MRI imaging to accurately measure aerosol deposition in the lung (H. Wang et al., 2016). A recent method using ultra-short echo (UTE) sequences and gadolinium-labeled aerosol resulted in an enhanced signal in the lung parenchyma, compatible with a precise quantitation of regional distribution of the aerosol deposition.

4. Methods to assess PK and toxicity after aerosol delivery

Although inhaled drug deposition and lung anatomy/physiology may be different between animals and humans, making extrapolation across species difficult, animal models are crucial to analyze the PD, PK and toxicity of aerosolized drugs. The methods used for pulmonary delivery, reviewed above, are critical because they involve variable drug losses in the delivery system and into the environment, specific sites of deposition (within or outside the respiratory tract), all of them altering therapeutic efficacy, PK and toxicity of the inhaled drug.

4.1 Determination of drug concentration in blood and lung environment

4.1.1 Determination of aerosolized-drug in the bloodstream

As drug concentration cannot be easily assessed in the lung, PK studies are done by measuring the levels of drug in the systemic circulation, both for local and systemically acting drugs. However, for drugs
acting locally, high serum concentrations are usually related to unwanted systemic exposure and potential safety concerns, and thus provide little information on efficacy. Anatomical lung differences, mucociliary clearance rates and the number of alveolar macrophages, which may all contribute to drug clearance, are known to vary significantly between species and may influence PK determination. PK studies assessing drug concentrations in blood are performed by collecting blood from a vein or alternatively via implantation of a catheter to avoid repeated venipuncture. Although NHPs are the closest to humans, with similar anatomy, physiology and genetics, rodent models are often used for preclinical PK studies. Nevertheless, mice are technically challenging as a species to undertake multiple blood sampling from lung dosimetry studies. In larger animals, multiple blood sampling is no longer an issue and allows a more accurate non-destructive profiling of the systemic serum/plasma levels in each animal. PK parameters describing the absorption, distribution and elimination of the drug and its bioavailability can be generated from appropriate mathematical methods (compartmental and/or non-compartmental analysis) and using the concentration measurements in plasma at relevant time points. However, indirect modelling of lung concentrations from drug concentration-time profiles in plasma using mathematical compartmental models, taking into account the delivery route may not be enough to predict the clinical outcome (Eichler and Müller, 1998; Paintaud, 2009; Presant et al., 1994). Indeed, many drugs, such as anti-infective agents, biotherapeutics and controlled release systems designed to prevent systemic exposure, do not distribute uniformly in different tissues and the dose-effect relationship is dependent on the distribution of the drug at the target site. Analyses of the drug concentration in the bloodstream may also be affected by the swallowed portion of the drug and subsequent absorption across the gastrointestinal tract. Clearance from the lung to the blood has been shown to be a linear function of the molecular weight of the drug regardless of the species (Matsukawa et al., 1997). Once in the systemic compartment, biopharmaceuticals like full-length immunoglobulin, with a molecular weight of 150kDa remain in the circulation for 3-4 weeks and are slowly metabolized. Monovalent fragments (e.g, single-chain antibody or scFv) have a blood clearance of ~10h with primarily renal excretion of 2-4h. Molecules with intermediate weight, ~70kDa (the threshold for glomerular filtration) have longer residence times. These discrepancies are therefore another strong reason for developing better methods to measure drug concentrations in the lung tissue to understand the distribution of drugs in the relevant anatomical compartment.

4.1.2 Determination of aerosolized-drug concentration in the lungs

Drug concentration can be measured in the airways and epithelial lining fluid (ELF) usually by bronchoalveolar lavage (BAL), a procedure which consists of injecting saline solution into the lungs, followed by re-aspiration. BAL can be performed on the whole lungs for small animals or portions of the lungs for larger species after euthanasia, or on live animals, usually non-rodents, via bronchoscopy (Mankikian et al., 2014). Because injection of solutions into the lungs interferes with local drug concentrations and thus PK parameters, BAL usually only gives useful information for one time point in one animal. However, it is possible in larger animals, to collect, throughout the experiment, several BAL samples from different respiratory tract regions, using bronchoscopy or tracheal swabbing through the nose (Jacobson et al., 2017). In this case, it is important to use an endogenous marker diffusing passively throughout the different tissue compartments as an internal reference (Kipnis, 2005). Alternatively drug concentration can be measured in the lung tissue, at autopsy, which requires resection, homogenization of lung tissue and extraction of the drug with the appropriate solvent. For
peptide-based therapies, the main drawback is that their lung residency may be hindered by their instability due to the presence of proteinases.

4.1.3 Imaging methods and microdialysis

Although imaging techniques are widely used to follow drug distribution, they are rarely employed for PK studies of aerosolized drugs in preclinical studies (Anderson, 2000; Fok et al., 1999; Guilleminault et al., 2014; Weinstein et al., 2012). Depending on the imaging techniques used, the drug is conjugated to the most relevant tracer, which involves labeling and purification of the drug conjugate. The major caveats for PK imaging include short tracer half-life that may limit kinetic follow up, in particular for biotherapeutics, such as antibodies with long half-life. Another limitation is the possible release of the tracer in vivo and passive diffusion into different tissues, and impairment of drug binding to its target antigen and/or transport receptor due to its conjugation to the tracer. Imaging techniques involve costly equipment and are associated with technical challenges to conjugate the drug, which limit their wider use for PK studies. We previously described the fate of a mAb, labeled with a fluorophore and delivered by the airways, using Near Infrared Fluorescent Imaging (NIRF). Although accurate quantification remains challenging, it was possible combining 3D in vivo imaging and 2D quantification on ex vivo lung specimens (Guilleminault et al., 2014).

In vivo microdialysis is an interesting and well-established semi-invasive sampling technique that has been used to study PK of drugs in various tissues (Brunner and Langer, 2006; Dhanani et al., 2010; Elmquist and Sawchuk, 1997; Zeitlinger et al., 2005). As this technique does not withdraw any body fluids or compromise homeostasis, it allows the repeated measurement of the unbound and/or soluble drug in the interstitial space from the same animal, which substantially reduces the number of animals required for preclinical PK studies. This method is based on the use of probes with a semi-permeable membrane, which are implanted in the tissue of interest and continuously perfused with a physiological solution (perfsusate) at a very slow flow rate (usually 1 to 10 µL/min). The implantation of microdialysis probes is possible under direct vision during thoracic surgery to avoid pneumothorax and collapse of the lung, and to date no major peripheral tissue trauma has been reported (de la Peña et al., 2000). The dialysate is collected at timed intervals and substances/drugs in the interstitial space fluid diffuse passively through the membrane pores, along their concentration gradient (Supplementary figure 4). Although, it has been used extensively to follow the kinetics of neurotransmitter levels in neuroscience research, and to monitor small drugs in peripheral tissues, it is now possible, thanks to higher molecular cut-off membrane and push-pull methods, to also analyze the fate of larger molecules, such as proteins. However, because the dialysate volume is small, in the microliter range, it requires highly sensitive methods for sample analysis. An additional limitation of microdialysis is the proper calibration of the microdialysis probes because the analyte diffusion is performed under non-equilibrium conditions and the concentration measured in the dialysate will reflect only a fraction of the concentrations in the interstitial fluid surrounding the catheter (Dhanani et al., 2010). Microdialysis has been shown to be feasible for most tissues, including the lungs, and in different species (de la Peña et al., 2000; Zeitlinger et al., 2005). Notably, it has been applied to the measurement of both non-infective and anti-infective agents with success, demonstrating its relevance for intra-bronchial PK measurement of drugs in the lungs (de la Peña et al., 2000; Zeitlinger et al., 2005). Microdialysis with monoclonal antibodies has recently been shown feasible (Jadhav et al., 2017).
4.1.4 Influence of pathological conditions on inhaled drug concentration in the lung

Disease states have to be taken into consideration for the accurate determination of drug deposition in the lung, especially those characterized by bronchoconstriction, inflammation and airway narrowing. Respiratory diseases, such as cystic fibrosis and chronic obstructive pulmonary disease exhibit changes in the architecture of the lung with obstruction of the airways, mucus hypersecretion and modification in the bifurcation angles, which may alter the distribution patterns of therapeutic aerosols, in particular those containing high-molecular weight protein therapeutics like mAbs. Pro-fibrotic diseases such as idiopathic pulmonary fibrosis or emphyzema, are characterized by the overexpression of proteases in the ELF, which may cause protein therapeutic degradation and hinder drug penetration to the epithelium. ELF also contains surfactant that have been shown to interact with exogenous proteins promoting their aggregations (Nag et al., 2007). Myeloid cells such as alveolar macrophages may also participate in the regulation of inhaled drug. Indeed, they have been described as barrier cells to the transport of large protein (>40Kda) from the airway into the parenchyma (Lombry et al., 2004). During the acute phase of lung inflammation, alveolar macrophages undergo rapid apoptosis, thereby reducing their interactions with inhaled drug. This may impact inhaled mAb clearance because alveolar macrophages express FcRn, which may prevent intracellular degradation of inhaled mAbs, after they deposited in the airways. Finally, inflammation injures both epithelial and endothelial lung barriers promoting leukocyte extravasation and infiltration of vascular fluid. This increased pulmonary microvascular permeability may dilute the drug within the lungs. On the other hand, inhaled drug leakage into the systemic circulation may occur if the bronchoalveolar barrier is disrupted. This was observed for an inhaled mAb, after an accidental acute lung injury due to gastric inhalation during an aerosoltherapy session (Guilleminault et al., 2014).

4.2 Scaling-up of animal results to determine first-in-man dose, for drug delivered by inhalation

First-in-man dose estimation from pre-clinical pharmacology data for systemically administered drugs is based from the common use of allometric dose scaling (Nair and Jacob, 2016). Allometric scaling approaches allows interspecies correlation with an empirical calculated pharmacologic parameter and is the standard way to approximate equivalent interspecies doses unless extrapolating doses based on other parameters is known to be more appropriate. For drug delivered by inhalation, scientific data to support the assumption that allometric scaling is useful to predict the dose is lacking. In a recent review (Phillips, 2017), allometric scaling exponent has be retrospectively calculated from experimental rodent data and the known inhaled clinical dose that needs to be deposited in the human lung. The author studied four inhaled compounds currently used clinically (mometasone, budesonide, salbutamol, ipratropium) and assumed that in all rodent, canine, nonhuman primate and human, the pulmonary deposition fractions were 10%, 25%, 30%, and 40%. The allometric interspecies scaling equation obtained was: \[ Y_h = Y_a \left( \frac{M_a}{M_h} \right)^b \] where \( Y_h \) is the human drug dose (μg), \( M_h \) is the human body mass (kg), \( Y_a \) is the animal drug dose (μg), \( M_a \) is the animal body mass (kg), and \( b \) is the allometric exponent. The average of the allometric exponents obtained in mouse, rat and human supports the current method of scaling using a fixed allometric exponent of 0.67 (Phillips, 2017). The utility of fixed exponent allometric scaling to project an inhaled human dose is pragmatic and could have a place in the drug development decision making process; however, it should be used cautiously as important simplifications were required (i.e. the
pulmonary deposition fractions) and this calculation being performed on a small dataset (n=4). Moreover, it may not be recommended for inhaled biotherapeutics, such as mAbs. Although the predictive value of allometry for mAbs is admitted with monkey data when mAb PK profile is linear, scaling up from animals to human is more complicated for mAbs with nonlinear PK because species differences (antigen density, antigen/FeRn interaction with mAbs, …) should be considered (Kamath, 2016; J. Wang et al., 2016) and require more mechanistic approaches (PKPD models, …).

4.3 Toxicology of inhaled biopharmaceuticals

Aerosolized drugs may potentially have adverse effects ranging from subtle changes in respiratory function to alterations in lung biochemistry, immunology, permeability and potentially mortality. The accurate determination of the dose reaching the lung is of particular importance, but as for pulmonary delivery or PK-PD studies, toxicology analysis of inhaled drugs will be susceptible to innate specificity of the animal model used (Figure 1), as well as the exposure system and the calculation method chosen.

As highlighted in Section I.B, it is generally accepted that the drug distribution throughout the lung will not be comparable to aerosol administration and that the toxicological profile of the aerosolized drug will be different to that of compounds administered intra-tracheally. Drugs or drug formulations are typically administered either as a nebulized solution/suspension, or as a dry powder (Owen, 2013). Currently, dry powder formulations comprise the majority of inhaled formulations under development. Due to the impracticalities of using clinical dry powder inhaler devices in toxicology studies, alternative aerosol generator devices have been designed for dosing of nonclinical species. Two systems are currently in use to generate dry powder aerosols for simultaneous dosing of typically up to 24 rodents in nose-only exposure cones or a variable number of dogs using facial masks: the Wright dust feeder and the relatively recent capsule-based aerosol generator (Armstrong et al., 2015; Paul et al., 2012). Regulatory studies based on Wright dust feeder always use aerosol administration, because this dosing method is considered closest to therapeutic dosing. In comparison, the capsule-based aerosol generator method is able to deliver similar lung doses over a large dosing range, but utilizes a lower amount of starting material and is therefore suitable for expensive compounds with limited available quantities (Paul et al., 2012).

The species used for regulatory toxicology studies are informed by regulatory guidelines, which require all assessments to be made in a rodent and non-rodent species, typically the rat and dog, although NHPs are also increasingly being used. It has been observed that certain forms of toxicity, both systemic and local, may be specific to the animal model and have little relevance for humans. Species-specific local toxicity or irritancy can often be observed in the nasal cavity of many experimental animals, especially obligate nasal breathers. This form of adverse event is typically not relevant for most inhaled products (i.e. pMDIs and dry powder inhalers) which are delivered via the oral cavity with no nasal exposure (Owen, 2013). However, nasal effects may be relevant for certain nebulized drugs for intended use in neonatal populations, as infants are also nasal breathers. Another adverse event often observed in rats, but not relevant for humans, is squamous metaplasia of the larynx. This local inflammation results from a high impaction of drug in the rat larynx following inhalation exposure, due to the nearly linear position of larynx behind the nasal cavity in rats. The immune response to the deposition may finally dictate the safety of the inhaled bioterapy.
Species-specific adverse events in the lung itself are rarer. It is widely recognized that rats are especially susceptible to lung overload with poorly soluble particulates. Lung overload occurs when the clearance mechanisms for removal of particulates, typically by macrophages, but also via particle entrapment in the mucus layer and removal via the mucociliary elevator, are saturated. Related to the phenomenon of lung overload in rats, is the effect of poorly soluble drug particles on alveolar macrophage populations. It has been reported that focal macrophage accumulations often with a “foamy” appearance are a frequent observation in non-clinical toxicology studies of dry powder formulations not only in rats, but non-rodent species as well (Forbes et al., 2014; Lewis et al., 2014). Above certain particle burdens, a progressive neutrophilic inflammation will develop with infiltration of inflammatory cells and elevated pro-inflammatory cytokine production. Chronic inflammation may lead to local tissue damage and fibrosis. In rats this can progress to neoplasia, although this has not been observed in humans or other nonclinical toxicology species (Owen, 2013; Wolff, 2015). The immunogenicity of biotherapeutics may also be an important issue. Anti-drug antibodies (ADA) may be generated and have detrimental impact on drug safety, efficacy and pharmacokinetics (Smith et al., 2016). Although the induction of ADA is in many cases harmless, serious consequences might occur, as shown in human, by the current epidemic of the antibody-induced severe anemia that is associated with the use of a specific form of recombinant erythropoietin (Schellekens, 2002). Structural alterations of proteins secondary to the inhalation process can potentially favor the generation of aggregates that could augment protein-specific immune response and eventually lead to the formation of ADA (Sauerborn et al., 2010). It is acknowledged that ADA formation in nonclinical species may not be predictive of clinical immunogenicity. However, it is accepted practice to assess whether differences in the PK/PD profile of the biopharmaceutical between the first and last dose occur or whether there is evidence of immune-mediated toxicity in nonclinical studies. In such cases, ADA investigations may be warranted (McElroy et al., 2013).

A further issue of relevance for the design of safety assessment studies for biopharmaceuticals is the impact of exaggerated pharmacology. Since the toxicity of many biopharmaceuticals is often related to their mechanism of action, for example hypoglycaemia following insulin administration, the dose increases routinely used in toxicology studies may induce a toxic pharmacologic response, which may be independent of the intrinsic toxicology. For this reason, ICH S6 guidelines require pharmacodynamic endpoints in nonclinical toxicology study design (McElroy, 2013). In such cases, the intrinsic and biological toxicity can and should also be assessed in the pharmacological species of interest, as well as animal models of disease. This can help to define whether the toxicity profile of the biopharmaceutical is primarily defined by intrinsic toxicity or exaggerated pharmacology (Cavagnaro, 2013).

5. Future directions and conclusion

Extrapolating data from animal studies to humans is a major challenge which is particularly true for inhaled medicines delivered through the pulmonary route. Because of the complexities and interspecies physiological, anatomical and metabolic differences in the respiratory tract, it can be difficult to bridge animal data to human patients inhaling a drug. This does not mean that animal models for aerosol drug delivery are irrelevant. They have contributed significantly to the development of all currently used pharmacological classes of drug that are delivered by topical administration to the lung, and they are helping with the development of
inhaled biotherapeutics. From a regulatory point of view they are mandatory, and both rodent and non-rodent (usually dog or NHP) species are required for this purpose. Rodents are widely used in preclinical studies and useful, in particular to decipher mechanisms of action considering the availability of a large number of tools/reagents and transgenic models, particularly in mice. However, it is now assumed that the expansion in the use of mice for PD studies, over the past decade, has not been associated with a marked increase in the number of effective new classes of inhaled drugs. The predictability of murine models is particularly questioned for biotherapeutics, and accordingly, there would seem to be an urgent need to reassess the use of the most appropriate non-clinical species, depending on the class of the drug, to predict safety and efficacy in man. Although there are ethical considerations, larger mammals such as NHPs may be more predictable for inhaled biologics being closer to humans, on a biochemical, cellular and immunological point of view. Moreover, those drugs often display a high specificity for their target raising further concerns on the relevant species to select for preclinical development.

The difficulty to translate promising drug candidates for inhalation from animal models to humans underlines the need for more predictive models. Although the utility of in vivo studies is sometime questioned, animals remain irreplaceable so far, because current in vitro ex vivo approaches still struggle to replicate the complex environment of the human respiratory tract and encounter similar limits as those of animal models when it comes to aerosol delivery. However, the progresses of the latest technologies are encouraging, offering great promises for the development of inhaled biotherapeutics. Among them, the following can be emphasized.

Airways epithelial cells cultured at the air-liquid interface reproduce a physiological respiratory epithelium and may be combined with a dose-controlled aerosol-cell exposure system (ALICE CLOUD) to evaluate transepithelial absorption of aerosolized protein therapeutic (Röhm et al., 2017). Additional applications with this delivery system may include the impact of inhaled biotherapeutics on airways epithelium integrity. Among them, the following can be emphasized. Airways epithelial cells cultured at the air-liquid interface reproduce a physiological respiratory epithelium and may be combined with a dose-controlled aerosol-cell exposure system (ALICE CLOUD) to evaluate transepithelial absorption of aerosolized protein therapeutic (Röhm et al., 2017). Additional applications with this delivery system may include the impact of inhaled biotherapeutics on airways epithelium integrity. Organ-on-chip technology recapitulates the dynamic tissue-tissue interactions on a microdevice. As an example, lung-chips integrate several tissue compartments (endothelium, epithelium) where cells are submitted to the mechanical and fluidic forces they experienced in the body, and can be exposed to exogenous agents (cigarette-smoke, pathogens,...) to mimic pathological conditions (Benam et al., 2016; Huh et al., 2010). Future developments to connect this biomimetic microsystem to aerosol delivery systems may be helpful to study the pharmacodynamics and PK parameters with inhaled biotherapeutics. Today, in silico approaches provide realistic 3D micro-models of the human respiratory system that can be combined with simulation techniques, like computational fluid dynamics, to explore respiratory airflows and aerosol transport in the lungs, useful to predict inhaled drug deposition (Hofemeier et al., 2017). Thus, hybrid modeling using these emerging technologies could be a solution to refine existent animal models of aerosol delivery.

Although inhalation is clearly not suited for all medicines and has to be chosen based on solid scientific arguments, it is time to reconsider the most judicious use of non-clinical species in the development of inhaled medicines to ensure improved rates of successful translation to the clinic. Rather than opposing in vivo models with other approaches, future challenges will be probably to implement complimentary approaches that more precisely recapitulate the human respiratory complexity and aerosol delivery in order to help future preclinical developments of inhaled biotherapeutics.
Acknowledgments:
This work was supported by a public grant overseen by the French National Research Agency (ANR) as part of the “Investissements d’Avenir” program (reference: ANR-10-LABX -53-01). It was also supported by two grants provided by Région Centre (MicroPulm and Flukiller).
References


https://doi.org/10.1016/j.cels.2016.10.003


European Medicines Agency Committee for Medicinal Products for Human Use, n.d. Guideline on the requirements for clinical documentation for orally inhaled products
(OIP) including the requirements for demonstration of therapeutic equivalence between two inhaled products for use in the treatment of asthma and chronic obstructive pulmonary disease (COPD) in adults and for use in the treatment of asthma in children and adolescents.


Marchand, S., Bouchene, S., de Monte, M., Guilleminault, L., Montharu, J., Cabrera, M., Grégoire, N., Gobin, P., Diot, P., Couet, W., Vecellio, L., 2015. Pharmacokinetics of
Colistin Methansulphonate (CMS) and Colistin after CMS Nebulisation in Baboon Monkeys. Pharm. Res. 32, 3403–3414.


Reichert, J.M., 2016. Antibodies to watch in 2016. mAbs 8, 197–204.


https://doi.org/10.1002/ppul.23509


Figure legends

**Figure 1.** Integrated scheme of advantages and limitations of animal models used to investigate aerosol drug delivery in pulmonary research. Anatomical and physiological features were obtained from (Asgharian et al., 2012; Crapo et al., 1983; Phalen and Oldham, 1983; Siegwart et al., 1971); animal pictures were obtained from Servier Medical Art.

**Figure 2.** Inter-species respiratory physiological differences. Respiratory rates (A) and tidal volumes (B) are expressed according to the body weight in several animal species (Asgharian et al., 2012; Bassett et al., 2014; Cryan et al., 2007).
Figure 2

A. 

B. 

Respiratory rate (breaths/min) vs. Weight (kg)

Tidal volume (ml) vs. Weight (kg)

Species indicated on graphs:
- Mice
- Rat
- Guinea Pig
- Rhesus Monkey
- Beagle Dog
- Large white pig
- Human

Graph A shows a decrease in respiratory rate with increasing weight, while Graph B shows an increase in tidal volume with increasing weight.
Table 1

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planar scintigraphy</td>
<td>Less expensive</td>
<td>Only 2-D representation of lung anatomy</td>
</tr>
<tr>
<td></td>
<td>Validated methods for quantification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Available standardized operating procedures</td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>Better resolution</td>
<td>Use of radio-labeled element</td>
</tr>
<tr>
<td></td>
<td>3-D representation of lung anatomy</td>
<td>Limited half-life of positron emitters</td>
</tr>
<tr>
<td></td>
<td>Co-acquisition with MRI/CT</td>
<td>Expensive facilities</td>
</tr>
<tr>
<td>SPECT</td>
<td>Better resolution</td>
<td>Use of radio-labeled element</td>
</tr>
<tr>
<td></td>
<td>3-D representation of lung anatomy</td>
<td>Long imaging time</td>
</tr>
<tr>
<td></td>
<td>Co-acquisition with MRI/CT</td>
<td>Complex quantification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expensive facilities</td>
</tr>
<tr>
<td>MRI</td>
<td>Promising technology, but still in development phase</td>
<td></td>
</tr>
</tbody>
</table>