



IN VIVO-IN VITRO CORRELATIONS FOR RESPIRATORY DRUG DELIVERY

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ABSTRACT

The focus of research now days has been expanded on respiratory system due to more area of nasal and pulmonary to deliver the pharmaceutical dosage form. The evaluation of two different marketed products can be obtained by determine the respiratory drug deposition rate and drug absorption in animal model testing. The tight monolayer cell junction barrier affects the absorption and deposition rate of drugs applied to respiratory system, when goes through *in vivo* animal testing. Droplet size of different product device such as nasal spray and nebulizer also affect the permeability of epithelial cell barrier. This study has focused on the *in vitro* and *in vivo* correlation of nasal drug delivery as well as pulmonary drug delivery system.

Keywords: *In Vitro* Testing, *In Vivo* Testing, Nasal Drug Delivery, Pulmonary Drug Delivery.

1. INTRODUCTION

The respiratory tract is a complex system, involving airways both inside and outside the thorax. The significance of the respiratory tract to drug delivery is that it offers two distinct drug delivery routes pulmonary and nasal. The pulmonary route is used for the delivery of bronchodilators and corticosteroids to treat patients with asthma and chronic obstructive pulmonary disease, while also providing a means of delivering a range of other locally acting drugs, notably inhaled antibiotics used by patients with cystic fibrosis¹. The nasal route is best known in the therapy of patients with rhinitis and sinusitis, where locally applied decongestants and corticosteroids are prescribed routinely². The nasal cavity is divided into two halves by the nasal septum and extends posteriorly to the nasopharynx, while the most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nostril. The respiratory region, the nasal conchae or turbinates, which occupies the major part of the nasal cavity, possesses lateral walls dividing it into 3 sections: the superior, middle and inferior nasal turbinates³. These folds provide the nasal cavity with a very high surface area compared to its small volume. The epithelial cells in the nasal vestibule are stratified, squamous and keratinized with sebaceous glands. Due to its nature, the nasal vestibule is very resistant to dehydration and can withstand noxious environmental substances and limits permeation of substances.

However, there is also the possibility to use either the lungs or the nasal cavity as a portal of entry for drugs to the body. Several systemically acting drugs given intranasally are already marketed, including sumatriptan, calcitonin and cyanocobalamin⁴. In addition, there has been much recent interest in delivering some drugs to the olfactory region in the roof of the nasal cavity, from which absorption direct into the brain may occur.

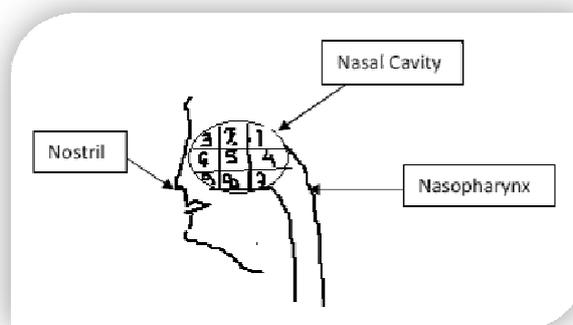


Figure 1: The nasal cavity is divided into a three-by-three grid, and the zones are defined as follows: Upper: 1 + 2 + 3; Lower: 7 + 8 + 9; Inner: 1 + 4 + 7; Outer 3 + 6 + 9.

It is hoped that systemically acting drugs given by the pulmonary route will soon be available. Therefore, in the context of conventional wisdom that the lung's alveolar regions are the most favorable for drug absorption, much of the efforts in an early stage of inhalation⁵ projects for systemic drug delivery are focused on generating aerosols in this size range. Unfortunately, this theory of aerosol deposition within the lung is largely inapplicable to small laboratory rodents, such as rats and mice, primarily due to their nose-breathing nature. Several companies are currently developing inhaled insulin products and a number of other systemically acting drugs are known to be in early stage development⁶.

The regulatory authorities place great importance upon data derived from *in vitro* tests of product performance, which include particle or droplet size distributions, emitted dose, spray pattern and plume geometry⁶. These tests can be performed accurately and reproducibly in the laboratory, which makes them excellent measures for purposes of quality control and product release. However, *in vitro* tests have an important limitation. They do not take into account either what happens when the spray interacts with the complex anatomy of the human

airways, or what happens when patients use pulmonary or nasal inhaler devices. Such information requires the assessment of drug delivery *in vivo*, which can be measured in either healthy volunteers or appropriate patient groups. Certain pharmacokinetic methods can *also* be used to quantify pulmonary drug delivery. In humans, orally inhaled aerosols greater than 5-10 μm are mostly trapped by oropharyngeal deposition and incapable of reaching the lung⁷. In contrast, smaller aerosols $\leq 5 \mu\text{m}$ can penetrate into the lung, although the finest aerosols $\leq 1 \mu\text{m}$ are mostly exhaled without deposition. This paper will review the relationships between *in vitro* and *in vivo* drug delivery data for both pulmonary and nasal drug route.

2. NASAL DRUG DELIVERY

2.1 In vitro testing

The United States Food and Drug Administration have issued draft regulatory guidance about the demonstration of bioavailability and bioequivalence for locally acting nasal products⁸. Measures of spray pattern and plume geometry allow the shape of the expanding spray cloud to be examined. Automatic actuation stations are preferred to avoid variations between operators. The rationale underpinning the use of *in vitro* tests for assessing bioavailability/bioequivalence for locally acting nasal products is that these tests can be performed reproducibly and show little variability, thus enabling any differences between two products to be detected readily. The rationale underpinning the use of *in vitro* tests for assessing bioavailability/bioequivalence for locally acting nasal products is that these tests can be performed reproducibly and show little variability, thus enabling any differences between two products to be detected readily. While the guidance recognizes the value of clinical endpoints, their use is discounted in BA/BE studies because previous data have shown difficulties in demonstrating differences in clinical response between two doses differing by an order of magnitude⁹. An additional *in vivo* study (preferably pharmacokinetic) is recommended for nasal formulations consisting of particulate suspensions. Traditional methods for assessing BE based on equivalent areas under the drug concentration versus time curve are considered inappropriate for locally acting drugs such as those given by inhalation to treat nasal or pulmonary diseases. Droplet size distribution may be measured by laser light diffraction¹¹, Nasal spray pumps typically have a D50 >50 μm , but with a broad spectrum of smaller and larger droplet sizes. Andersen cascade impactors may also be used to quantify size distributions from nasal sprays¹².

2.2 In vivo methods

2.2.1 Cell culture models of the respiratory epithelium

The structure and function of the lung and respiratory epithelium have been reviewed extensively from a biopharmaceutical perspective¹³⁻¹⁵. The airway and alveolar epithelium of the lung provide the principal

physical barrier to drug absorption and are composed of different cell types and have distinct properties. The general rationale in modeling the epithelium has been to develop monocultures based on the cell type contributing the majority of the epithelial surface area. This corresponds to columnar cells in the airway and the alveolar type I cell in the alveolar region. The selection of cell type to model the epithelium is restricted by the desirability of using human cells and the requirement that the cells form functional tight junctions in culture¹⁷. The convenience and practical benefits of using cell lines, if suitable cell lines are available, must be balanced against any inherent differences to native cell phenotype. Knowledge of all three aspects is required for the rational design of an inhaled medicine, but recent advances in device design and formulation strategies are outstripping our understanding of how inhaled medicines interact with the lung. Two human-derived cell lines, 16HBE14o-cell and Calu-3-cell, are recognized to exhibit suitable respiratory bronchial epithelial cell-like phenotype and to acquire suitable barrier properties in culture¹⁸. For the alveolar region, no suitable cell lines are available and isolation and culture of alveolar type II cells to ATI-like human alveolar epithelial cell monolayers is necessary²¹. The morphology of 16HBE14o-cells, Calu-3-cells in culture generally corresponds to that of the native epithelium, with Calu-3 cells appearing to be of a columnar phenotype, 16HBE14o-cells being cuboidal in shape and epithelial cells being much flatter and laterally extended.

2.2.2 Airway epithelial cells

The surface of the airways is lined by a pseudostratified epithelium. The air-interfaced cells are predominately ciliated columnar cells interspersed with goblet cells in the upper airways and cuboidal ciliated cells interspersed with Clara cells in the lower airways. Normal human bronchial epithelial cell culture can provide a mixed ciliated and goblet cell culture model of the airway, which has been reported to provide a permeability barrier which would be suitable for drug transport studies²²⁻²⁴. Pre-cultured human tracheobronchial cell layers composed of well-differentiated ciliated and goblet cells are also commercially available as the EpiAirway system from the Mattek Corporation²⁵. Although the EpiAirway is marketed and used for drug delivery purposes, the commercial nature of these applications means that little data has been reported²⁵. In general, primary culture is less convenient and economic than the use of cell lines and the primary co-culture cell layers have tended not to be used for biopharmaceutical purposes. 16HBE14o-cells are transformed bronchial epithelial cells from a 1-year old heart lung transplant patient and form confluent layers with differentiated epithelial morphology and functions²⁶. Calu-3 cells are derived from a bronchial adenocarcinoma of the airway and form confluent mixed cultures of ciliated and secretory cells²⁷. Culture methods and properties of these cell lines have been described in reports of studies that aimed to establish the barrier properties of the cells to solute transport²⁸. But it should



be noted that culture methods vary between laboratories. The influence of air-liquid interface or submerged culture appears to be one of the major influences on the transepithelial electrical resistance.

Traditional reliance on the use of animals and animal tissue preparations to evaluate the fate of inhaled substances³⁰ is declining. In 1959, Russell and Burch published 'The Principles of Humane Experimental Technique'. They proposed that if animals were to be used in experiments, every effort should be made to replace them with nontoxic alternatives, to reduce to a minimum the number of animals used. Radionuclide imaging studies have been used to quantify nasal cavity deposition and clearance, to look for correlations between drug deposition and subsequent blood levels of systemically acting drugs, for quantifying how much drug reaches specific regions of the nasal airways such as the olfactory region and the sinuses³⁵ and to demonstrate negligible lung deposition for formulations delivered by spray pump devices³⁹. Quantification of nasal drug delivery is made easier if the subject also undergoes a magnetic resonance imaging (MRI) procedure of the head. The MRI scan and radionuclide image can then be co-registered, enabling the location of deposition areas on radionuclide images to be assigned more readily to specific anatomical sites. As will be described, a ventilation scan with a radioactive inert gas (^{81m}Kr or ¹³³Xe) can also be used to outline the nasal cavity and hence provide anatomical reference points.

2.3 In-vitro/in-vivo correlation for nasal drug delivery

The relationship between *in vitro* laboratory test data and *in vivo* radionuclide imaging study has been investigated in several studies, generally to assess the extent to which *in vitro* results can predict *in vivo* product performance. In one study³¹, a comparison was made between a nebulizer (Hudson Updraft II) and an aqueous nasal spray (Beconase AQ). The droplet mass median diameters were 6 mm and 79 mm, respectively, indicating that these products had clearly different properties when tested *in vitro*⁴³. The nebulizer was primarily a device for pulmonary delivery, but was adapted for nasal administration. The devices were then compared *in vivo* in seven healthy subjects and in one asthmatic patient. The formulations were radiolabeled by the addition of ^{99m}Tc. The intranasal distribution patterns were determined for nebulizer and pump Spray. The outlines of the nasal cavity were determined from a ventilation scan with a radioactive inert gas, and the nasal cavity was divided into a three-by-three grid (Fig.1), comprising "upper," "lower," "inner" and "outer" zones. The inner zone/outer zone and upper zone/lower zone deposition ratios were calculated for the ^{99m}Tc images. As with the *in vitro* data, there were clear differences between the two products, with the smaller droplets from the nebulizer penetrating more readily into the inner and upper regions of the nasal cavity, leading to higher inner zone/ outer zone and upper zone/lower zone deposition

ratios. In addition, means of 33% and 58% of the delivered dose was recorded in the lungs of two subjects who inhaled from the nebulizer, while lung deposition was negligible when the same subjects inhaled from the pump spray⁴⁴. The study showed that for two markedly different products, such as a nebulizer and a pump spray, differences in drug delivery measured *in vitro* were reflected in differences in drug delivery derived from *in vivo* testing.

The *in vitro* data showed small, but statistically significant, differences in several *in vitro* test parameters, which could have led to the conclusion that the products were not equivalent. For *in vivo* testing, the formulations were labeled, and the regional distributions within the nasal cavity were assessed from inner zone/outer zone and upper zone/lower zone deposition ratios as in the case of the earlier study where nebulizer and pump spray were compared⁴⁵. The inter-subject coefficients of variation of deposition parameters were high, and hence, there were no differences detectable between the two pump sprays in terms of their regional deposition patterns. Inner zone/outer zone and upper zone/lower zone deposition ratios were higher for pump in some subjects, and higher for pump in other subjects.

3. PULMONARY DRUG DELIVERY

Pulmonary drug delivery has a rich history that can be traced back to the writings of many ancient civilizations. The modern era of inhaled drug delivery dates from the first part of the 20th century, when inhaled adrenaline was first given to asthmatic patients, and when both inhaled antibiotics and inhaled insulin were tried for the first time. The success of pulmonary drug delivery depends upon depositing enough drug aerosols, either as solid particles or liquid droplets, at the correct site within the bronchial tree⁵. Particles or droplets smaller than 3 mm diameter are considered the ones most suitable for penetration to the alveolated regions in the deep lung, which is the target site for inhaled peptides and proteins⁸.

3.1 Animal models for in vivo study

The evaluation of factors influencing the delivery of drugs across the airways should be conducted in healthy or diseased humans. Animal models, however, a large number of studies have employed rodents, but other animal models including rabbits, dogs, sheep, monkeys and non-human primates have also been used. Initial studies in virtually every area of pharmaceutical and pharmacological research have been performed in small rodents such as mice, rats and guinea pigs because terminal procedures can be easily conducted and large numbers of these animals can be used for statistical validity¹⁵. Rats and guinea pigs are frequently used for the study of drug delivery to the lungs because a variety of dosing techniques that require a small amount of the drug can be employed and they are good models for a number of respiratory disease states. Therefore guinea pigs are regarded as a good model of



bronchoconstriction/bronchodilation in the evaluation of drugs used to treat asthma.

Hormonally and immunologically guinea pigs are more like humans than other rodents; thus they are often used to model human infectious diseases¹⁸. Although rats are not as similar to humans, they have been used to study a range of diseases such as emphysema¹⁹, influenza²⁰ and pulmonary fibrosis²¹. Delivery of large doses of aerosol to mice is difficult to achieve in short periods of time and the amount of biological fluids that can be collected is small. Nevertheless, mice have been used to investigate drug delivery in disease states such as cystic fibrosis²³ and lung cancer²⁴. The use of larger species such as rabbits, dogs, sheep and primates is considered when the study design is more complex, or when administration of larger doses of drug or collection of larger amounts of body fluids over longer periods of time is required. However, regulatory agencies and cost may limit the number of animals employed in these studies, which in turn may influence the statistical analysis. The dog model has been utilized to assess delivery of insulin²⁵ and the treatment of some allergies²⁶, monkeys and non-human primates have been used to assess vaccine delivery²⁷ or drugs to treat cystic fibrosis²⁸, while sheep are considered a good model of lung injury²⁹.

3.1.1 Rational model

Although many species are suitable for studies of disposition of drugs, in and from the lungs, the organization of cells and tissues in the lung is an important consideration in the selection of an animal model, when the results are to be extrapolated to humans. This is crucial when the quantitative description of airways may be used in mathematical models for predicting efficiencies of deposition and clearance of inhaled drugs. Comparisons of the morphologic characteristics of the lungs of many mammalian species have been reported³⁰ and key differences in individual anatomical parts are discussed below. Some of the anatomical characteristics of the lungs for several species are compared to those of the human lungs as revealed on replica casts³⁰. In overall shape, the human lung tends to be more spherical than the lungs of other species. The significance of overall shape is seen in the relatively symmetrical airway branching scheme at essentially all levels in the human compared with the long tapering monopodial airways with small lateral branches characteristic of all the other species.

In terms of the number of lobes, the left human lung is divided into two lobes, the superior and inferior, and the right lung is divided into three by the addition of a middle lobe. Laboratory mammals including non-human primates are divided into four lobes. The left lungs from mice and rats are not divided but those from larger mammals such as guinea pigs and rabbits are divided³¹. Several studies have indicated that the variations in airway branching patterns and the consequent effects on airflow across species can contribute to differences in regional

deposition in the lungs^{31,32}, but this must be seen in the context of other contributory factors e.g. differences in breathing patterns, obligate nose-breathing and the physics of the aerosols delivered³². Overall it would appear that the airway anatomy and oral breathing of human's leads to a greater amount of upper bronchial airway particle deposition and to greater deposition on localized surfaces near airway bifurcations compared to smaller mammals³⁵. Compared with the other species, human lungs contain greater number of macrophages. Significant interspecies differences in diameter, volume, number and functionality have been reported between human, monkey and rats/hamsters alveolar macrophages³⁷⁻⁴⁰, which may have implications for effective clearance. The thickness of the interstitial and the pulmonary capillary endothelium of the human lungs were also significantly.

3.2 Methods of aerosol administration

The efficacy of an inhaled drug is influenced by the amount of drug deposited at the target site. However, care must be taken when interpreting and comparing aerosol delivery efficiency data. For example, studies may report the efficiency of delivery in terms of the delivered dose, but this parameter does not consider the fraction of the dose deposited in the periphery of the lung that is required to exert action locally or the absorbed dose, as in the case of drugs intended for systemic action. The method of aerosol administration is a key factor in the design of animal studies for drug delivery to the lungs and will impact on the accuracy of the results obtained. In inhalation toxicology there are methods to deliver accurate aerosol doses to conscious animals by passive inhalation³⁸, but their use to assess drug delivery may be impractical due to their complexity and the requirement for large reservoirs of drug, which are not relevant to small bolus doses. Direct administration methods are a good alternative, since a finite amount of material can be delivered to animals. However, since oropharyngeal and/or nasopharyngeal deposition are avoided in this approach, the site of lung deposition is complicated by altered dependence on droplet or particle size effects compared with aerosol systems. Both methods of administration are discussed below.

3.2.1 Direct administration methods

The main advantages of direct administration are that small and relatively large doses of drug can be delivered by these methods and that the dose delivered can be accurately measured. The main disadvantage is that the use of these methods for multiple or consecutive dosing may not be recommended, since they are performed under anesthesia, and the insertion of the device may cause local irritation. Direct administration methods include liquid instillation, spray instillation and dry powder insufflations, but only the last two deliver drug in a form proxy to true aerosol. In general, they involve visualization of the trachea of the animal with the help of a laryngoscope to place a thin stainless steel tube in the



trachea, near the carina, to administer drugs. For small rodents, the procedure is straight-forward but for large animals, including rabbits, dogs, pigs and monkeys; some surgical procedures such as tracheotomy may need to be performed to accommodate the device. Liquid instillation delivers drugs in the form of a liquid bolus. This method can cause significant stress in the subject, prevents uniform distribution throughout the lung and an unknown amount of the drug may be coughed up or swallowed. The atomizer in the tip of a long, thin, stainless steel tube generates a plume of liquid aerosol that can be deposited in the airways and deep lung. The powder is then dispersed by applying small "puffs" of air to the device using an empty plastic syringe and the amount of sample delivered to the lungs can be determined by gravimetric analysis. The insufflators have been used to deliver different powders ranging from nanoparticles³⁸ to large porous particles.

3.2.2 Passive inhalation

Experiments designed to deliver aerosolized drugs to conscious animals by passive inhalation employ exposure chambers that can be for whole-body, head-only and nose-only⁴⁷. Each exposure type has its set of advantages and disadvantages. Advantages of the whole-body inhalation systems include the ability to expose animals without restraint. The major disadvantage of these systems is the possibility of drug absorption by other routes including oral and percutaneous. A modified whole-body exposure chamber was made using a conventional metabolism chamber with a polypropylene tube inserted to restrain the animal³⁷. This chamber has the advantage of a small volume sufficient for the animal to receive a large dose of aerosol based on its inspiratory flow. Significant inter-individual variation might be anticipated as animals will vary in size, lung capacity and breathing parameters. Solution and suspension aerosols have been characterized and shown to retain the median particle size and distribution generated by the nebulizer after passing through the chamber³⁸.

3.3 Methods to assess deposition of inhaled drug

3.3.1 Determination of local and systemic drug concentration

Once the drug has been delivered to the lungs, it is quantified by various methods depending on its desired effect. In studies involving delivery of drugs for local action, determination of drug concentrations in the lung environment (epithelial lining fluid and tissue) together with plasma concentrations provide more valuable information. However, since the procedure is terminal for most animal models, large numbers of subjects are required making these studies costly and complicated by regulatory issues. Bronchoalveolar lavage (BAL) is the sampling of the lower respiratory tracts by instillation of sterile saline and subsequent aspiration of the fluid³⁹. In human subjects BAL is performed under mild sedation. A flexible fiberoptic bronchoscope is employed to pass as far as possible in the right middle lobe or left upper lobe

of the lung. Normal saline solution is introduced and aspirated. Then, the aspirated fluid is collected and analyzed. In large laboratory animals such as dogs and non-human primates, *in vivo* BAL is performed in a manner similar to that used in humans. BAL of small laboratory animals can be performed *in vivo* if required⁴⁰, but most lavage of rodents are performed on excised lungs. A catheter inserted into the trachea is used to instill the saline solution. Lavage volumes also vary for small laboratory animals, but usually the lavage volumes are approximately half of the total lung capacity of the section of the lung lavaged.

To evaluate drug concentration in BAL, two to four lavage might be performed to avoid excessive dilution of the drug. The concentration of urea in the BAL can be used as an endogenous marker⁴¹ to estimate the sample dilution and accurately determine drug concentrations. Quantification of the drug in lung tissue can also be performed, but may be challenging due to complicated extraction procedures that may compromise the integrity of the drug. Determination of drug concentrations in tissue may also provide information on drug metabolism in the lung, such as those reported for insulin⁴². For drugs intended for systemic action, concentrations are determined in serum or plasma. The blood collection sites are determined based on the volume and frequency of sampling required. In pharmacokinetic studies conducted in small rodents that require frequent blood sampling for a short period of time (usually less than a week), implantation of a cannula in the jugular vein is recommended. This requires a relatively complex surgical procedure. For studies that require less frequent sampling, localized bleeding in tail vein or artery, saphenous vein and the dorsal metatarsal vein can be used. In rabbits, the central ear artery and marginal ear veins are commonly used⁴³. In large animals, blood may be collected from a superficial implanted catheter from the femoral, cephalic or jugular veins^{44,45}.

3.3.2 Pharmacokinetics models for the disposition of drugs in the lungs

The pharmacokinetics of drugs after pulmonary delivery depends upon the dynamic interaction of different factors such as the site of deposition, clearance mechanisms, drug formulation (solid or liquid), dissolution rate, drug metabolism and mechanism of absorption⁴⁹. In animals as in human subjects, the factors that will influence drug deposition are particle shape, size and distribution, hygroscopicity, static charge, anatomy of the airways and breathing patterns such as frequency and tidal volume. However, as reviewed in previous paragraphs, the two latter factors are different in animals, thus, different deposition patterns should be expected in the different animal species that may influence the pharmacokinetic of drugs. Clearance mechanisms include mucociliary clearance and endocytosis. Large interspecies differences have been reported in the lung clearance of inhaled particles^{51,52}. However, lung models have been developed



to extrapolate between different species⁵⁴. Studies have also shown that the transport of particles by macrophages to the larynx that is substantial in rodents is rather small in humans. Extensive reviews have been published on mechanisms of drug absorption in the lungs. Few models have been proposed to address lung residence time of soluble aerosols, due to the complexity of the processes involved and the factors that affect these. Byron developed a mathematical method to determine residence times in the various regions of the respiratory tract as well as maximum deposition in these regions based on optimization of modes of inhalation and particle size. However, this model does not differentiate between drug released (pharmacologically active) and unreleased drug does it separate absorption rate from dissolution rate. Therefore, Gonda⁵⁵ proposed a compartmental model that simulated the effects of release rate and multiple dosing on the duration of 'free drug levels' in the respiratory tract.

This model also accounts for the possibility of accumulation of carrier materials during chronic administration as a function of drug release rate. Pharmacokinetic parameters after aerosol administration of a drug can be calculated using compartmental or non-compartmental methods, and recently physiologically-based PK models are being used more widely. Compartmental methods consider the body as a system of compartments that usually is not physiologically or anatomically significant and determines the drug distribution between these compartments⁵⁶. Compartments are arranged in a mammillary model and evaluated by inter-compartmental transfer constants that assume instantaneous distribution. It is assumed that the rate of transfer between compartments and the rate of elimination of drug from a compartment follow first order or linear kinetics. The criteria to determine the best fitting model to a specific set of data are the Akaike criteria, the model selection criteria, the coefficient of variation, and the width of the confidence interval for each parameter estimate. The systemic disposition of most inhaled drugs is best characterized by one or two-compartment models. The disposition of a few drugs has been characterized by a three compartment model with central elimination. Non-compartmental methods consider a central homogeneous space, where drug input and elimination occur, and a heterogeneous space⁵⁴. Drug is also sampled in the central space and the change of drug concentration over time in this space is usually regarded as a statistical distribution curve⁵⁵.

These methods are usually based on the estimation of the area under the drug concentration-time profile. Clearance, bioavailability, mean residence time and mean absorption time can be calculated by this method. The main contribution of physiologically-based pharmacokinetic methods is that they allow the prediction of drug concentrations in tissues in which samples cannot be easily collected. These models are well suited for making high-low dose, dose route and

interspecies extrapolations by incorporating quantitative descriptions of the physiological and biochemical processes of the animal and the biochemical properties of the drug⁵⁶. A classical PBPK consists of different compartments with each representing a particular organ.

3.3.3 Methods to calculate the rate of absorption and pulmonary bioavailability

The rate of absorption has been determined by measuring the disappearance of drugs from the lungs or their appearance in systemic circulation. The rate of absorption is then calculated after curve fitting of semi-logarithmic plots of the % remaining in the lung versus time or drug concentration over time⁵⁷. The estimation of pulmonary rate of absorption is complicated by different situations such as gastrointestinal absorption of drug swallowed following pulmonary administration, lack of sensitive methods to detect relatively low plasma and urine drug concentrations achieved under these circumstances, and pulmonary metabolism. The three methods most commonly used to estimate the rate and extent of absorption is the Wagner-Nelson, the Loo Riegelman and the observational methods. The first two are based on curve fitting by compartmental analysis whereas the observational method is based on non-compartmental analysis. The most important limitation of the Wagner-Nelson method is that it is applied exclusively to drugs with one compartment disposition⁸. Loo Riegelman proposed a modification in which the amounts of drugs absorbed at specific times could be calculated using two-compartment body models with first order elimination processes⁵⁷⁻⁵⁸.

An inherent limitation of this method to determine pulmonary absorption is the intra-subject variability in inter-compartmental transfer constants between intravenous and pulmonary administration. The observational method is the most simple and practical method. C_{max} and T_{max} parameters, obtained directly from drug concentration versus time plots, are used to determine the rate of absorption. C_{max} depends on both the rate and extent of absorption while T_{max} takes discrete values and may be subject to reliability problems because its frequency is not normally distributed. Alternative measures include C_{max} / AUC_{∞} , C_{max} / T_{max} , C_{max} / AUC_{max} . Caution should be exerted when interpreting the constant of absorption calculated for a drug delivered by the pulmonary route, since some characterization. The term bioavailability describes the rate and extent of drug absorption. Methods for assessing bioavailability of drugs in the lungs are limited by analytical issues related to the low drug concentrations in lung tissue and plasma after aerosol administration. For some drugs such as β -agonists the issue of low plasma concentration can be circumvented by measuring drug in urine.

Oral charcoal has also been used to prevent GI absorption of drug that is swallowed after inhalation. Complementary techniques such as imaging can be also used to estimate drug bioavailability. Pulmonary



bioavailability can be calculated using AUCs after IV and pulmonary administration using the following equation.

$$F = \frac{\text{AUC } 0 \rightarrow \infty \text{ lung Dose}_{\text{IV}} * 100}{\text{AUC } 0 \rightarrow \infty \text{ IV Dose}_{\text{lung}}}$$

3.4 Imaging of drug deposition and absorption

Imaging is an exciting tool in drug development and its role in the assessment of drug delivery is growing. With the improvements in imaging technology it is now possible to determine the delivery efficiency of a drug to its site of action and the resulting pharmacokinetics. Being able to characterize these parameters using non-invasive imaging of whole animals is particularly useful for assessment of inhalation delivery systems given the complex anatomy of the lungs and the complex biophysics governing aerosol deposition. Drug distribution and kinetics of inhaled drugs in human lungs are primarily assessed by the imaging of radiotracers. Gamma scintigraphy is the most commonly used imaging method, but single photon emission computed tomography (SPECT) and positron emission tomography (PET) are growing in popularity⁴⁹. In general, two major approaches have been adopted for imaging small animals: the first is the adaptation of clinical imaging technologies such as gamma scintigraphy, SPECT and PET for smaller animal dimensions, and the second is based on new evolving technologies based on whole-body photonic imaging.

Different formulations and devices on drug deposition and clearance in the lungs affected drug absorption. There are difficulties and limitations to its use that include a need for careful validation of the tracer and potential problems in trying to quantify distribution with what is essentially a non-quantitative technique⁵⁰. The technology behind human scanning has improved greatly over recent years, particularly with the emergence of SPECT and PET scans. SPECT is essentially planar imaging with a different gamma camera. In essence, planar images are acquired from several different angles and used to construct a 3-D distribution of radioactivity within the lungs and clinically is becoming more popular than planar imaging, due to the improved data quality from both a qualitative and quantitative perspective. The disadvantages of this technique include the extra time to complete a scan and labeling of the drug of interest⁵¹. PET is a 3D functional imaging technique that provides accurate information on dose, distribution and kinetics of an inhaled radiotracer in the lungs. This technique used to assess drug delivery to animals would provide a wealth of additional information including more accurate images detailing the regional distribution of the drug in the lungs.

3.5 In-vitro/in-vivo correlation for pulmonary drug delivery

A basic consideration in evaluating the respiratory cell culture models is their permeability compared to that of native airway and alveolar lungs. Values are not available for human lungs, but TEER and mannitol permeability

have been reported for animal respiratory epithelium. The TEER of rabbit airway epithelium has been reported to be 260-300 U cm²⁵⁰, which is exceeded by the TEER of the 16HBE14o-cell and Calu-3 cell layers shown in. For comparison, TEER for well-differentiated primary cultured human tracheobronchial cells has been reported in the ranges 300-500²⁴ and 450-650 U cm²²⁵. Encouragingly, where data is available for the permeability of airway tissue to mannitol (in dog and guinea pig²² values are comparable to permeability in the 16HBE14o-cell and Calu-3 cell models, while the lower permeability of isolated rat lung, which is thought to reflect the alveolar region. The first *in vitro-in vivo* comparisons of permeability in airway cell models with absorption from the intact lung have been performed. The availability of *in vivo* data is limited, and the variation in methodology used in pulmonary absorption experiments (e.g. species, administration by aerosol or solution, measurement of blood levels or pulmonary retention), restrict the availability of coherent data sets. *In vitro-in vivo* correlation has been reported for Calu-3-cells⁴⁰ by comparing solute permeability in cell culture to data obtained mainly by Schanker and co-workers in the 1970-1980s using disappearance of intratracheally.

Administered compounds in rats. The work of Tronde et al has recently provided *in vivo* and *ex vivo* data for absorption from rat lungs for several compounds. Permeability in the non-respiratory Caco-2-cell line provided a good correlation with absorption from the rat lung and these studies are being extended to measure the permeability of the same compounds in 16HBE14o-cells. For the hAEPc model *in vitro- in vivo* correlation has not been reported. Effros and Mason showed an apparent inverse relationship between molecular weight and rate of lung clearance of molecules of different sizes *in vivo*. This relationship has been found in alveolar epithelial models, with similar data being reported in rat^{52,52} and human monolayer. A further illustration of the 'leakiness' of A549 cells is that other epithelial cell models are highly restrictive to the transport of large molecules such as FITC-dextran 70,000. The high permeability of A549 cells is a result of the cell line being functionally deficient in tight junctions and the excessive 'leakiness' of A549 cell layers has been recognized previously. It was the use of 'tight' monolayers that allowed the permeability of proteins across the rat ATI-like cells to be recognized as being higher than predicted by their molecular size⁵¹. These observations point to transcytosis involving caveolae and clathrin-coated pits being the likely route of alveolar epithelial protein transport and, intriguingly, these actively absorbed proteins were processed relatively intact, whereas other proteins undergo significant degradation⁵². In general, the absorption of passively transported compounds can be predicted using non-lung cell culture models, with *in vitro-in vivo* relationships reflecting the 'tightness' of the respective intercellular routes which progressively restrict the transport of larger and more hydrophilic compounds. The



use of respiratory cells will, however, be crucial for the study of lung-specific drug transport or metabolic pathways. As respiratory cells are the target for inhaled formulations or particles, it will also be important to use these cells to evaluate drug targeting strategies.

In vitro particle size data and *in vivo* lung deposition data are often obtained concurrently for inhaled products and this provides a bank of data from which to examine relationships between the results of *in vitro* and *in vivo* methodologies. In a meta-analysis involving 18 different inhaler systems, correlations were sought between *in vitro* and *in vivo* data⁵⁸. Lung deposition was assessed by gamma scintigraphy, using optimal inhalation modes for each device. It was found that whole lung deposition and FPF (by multi-stage liquid impinger) were correlated, but that for all inhalers tested, whole lung deposition was overestimated by FPF, regardless of whether the data were expressed as the percentage of the dose associated with particles smaller than 5 mm diameter, or as the percentage of the dose recovered from the lowest impactor stages. Interestingly, a better correlation was obtained between whole lung deposition and the percentage of the aerosol mass contained in particles smaller than 3 mm diameter. There is evidence that the discrepancy between *in vitro* data and *in vivo* data may be less marked.

4. CONCLUSION

The considerations described above, whether applied to pulmonary or to nasal drug delivery, indicate that, the correlations does exist between *in vitro* and *in vivo* drug delivery data. The study has proved that, the deposition rate is inversely proportional to particle size distribution and droplets of different pharmaceutical device products of respiratory system. The whole lung and nasal deposition and percentage of aerosol mass contained in particles less than 3 µm diameter. The penetration of drug of less than 3 µm particle sizes is more up to the deep lung area, which is real targeted site of drug delivery. Animal isolated cell model shows that high permeability to those area where the lackness of tight junction. This is particularly true for systemic delivery across the lung mucosa where the impact of normal lung perfusion, the complete epithelial barrier and clearance mechanisms are significant and can only be modeled in whole animals. So *in vivo* deposition measurements for novel pulmonary or nasal delivery system may be useful adjunct to *in vitro* testing since they allow drug delivery to be assessed under situations closure to those pertinent during clinical use and are likely to provide better for caste of clinical effect.

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