Methods used to assess pulmonary deposition and absorption of drugs

Cary Mobley and Günther Hochhaus

The assessment of pulmonary drug absorption and deposition is becoming increasingly important in drug development. Absorption information can be used to maximize pulmonary selectivity, to screen drug candidates and to help evaluate the bioequivalence of generic inhalation products. Several methods are available to investigate pulmonary drug absorption and deposition, ranging from *in vitro* experiments to *in vivo* pharmacokinetic and pharmacodynamic analyses. In combination, these methods can indicate the fate of an inhaled drug.

Cary Mobley
College of Pharmacy
Department of Pharmaceutical Sciences
Nova Southeastern University
Fort Lauderdale
FL 33328, USA
*__Günther Hochhaus__
College of Pharmacy
Department of Pharmaceutics
University of Florida,
Gainesville
FL 32610, USA
*tel: +1 352 846 2727
fax: +1 352 392 4447
e-mail: hochhaus@ufl.edu

▼ For the development of pulmonary inhalation products, the assessment of drug deposition and absorption provides important information for evaluating drug candidates. The amount of drug that enters the lung, the location of deposition, the residence time at deposition sites and absorption into the systemic circulation can all provide crucial knowledge for the formulator. This knowledge can be important for determining pulmonary selectivity for locally acting drugs (e.g. β agonists) or for determining the bioavailability of systemically acting drugs (e.g. proteins and peptides). In addition, generic inhalation drugs are currently in development and the FDA needs to create approval guidelines that will encompass the effects of deposition and absorption on the bioequivalence of these products. Here we describe some methods of evaluating pulmonary drug absorption and deposition, with a focus on evaluating the pulmonary absorption of drugs delivered to the lung as aerosols for the treatment of respiratory disease.

Basic factors affecting the fate of the inhaled drug

The predominant methods of delivering pulmonary drug aerosols to patients are propellant-driven metered-dose inhalers (MDIs), dry powder inhalers (DPIs) and nebulizers (compressed air and ultrasonic). These devices create respirable clouds of drug product. The drug in these aerosol clouds will either be in the solid state (e.g. DPIs and many MDIs) or in the dissolved state (e.g. nebulizers). The pulmonary fate of the aerosolized drug is influenced by where the aerosol particle is deposited in the lung (central or peripheral regions of the lung) and by how fast the deposited particles dissolve.

When an aerosolized drug product is orally inhaled, aerosol particles will travel to different regions depending on factors including lung morphology, breathing pattern, aerosol velocity and geometry, particle size and particle density. As the particles move through the lungs, they can deposit at any point from the mouth to the conducting airways to the alveoli. A primary determinant of the location of particle deposition is particle size, which is generally characterized by the aerodynamic diameter (AD), and takes into account such factors as particle diameter, shape and density. Large particles (AD >5–6 μm) are unable to stay in the moving air stream and therefore tend to inertially impact in the mouth, throat and at points of bifurcation in the first generations of the conducting airways. As the air stream moves deeper into the lungs, the velocity slows, and smaller particles (AD ~1–5 μm) fall out of the air stream and deposit primarily by gravitational sedimentation. Gravitational sedimentation is facilitated by retaining the aerosol cloud for as long a time period as possible by holding one's breath and can occur deep into the respiratory region of the lung. The smallest particles (AD <0.5–1 μm) will continue to follow the air stream to its terminus and will either deposit through random motion (Brownian) or will be exhaled. For humans,

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the ideal particle size range to achieve the broadest and deepest penetration into the lungs is ~2–3 μm (Refs 1–3).

Environments for drug absorption in the lung

The structure of the respiratory tract is well known[4]. Throughout the lungs, an inhaled drug particle will first deposit in a thin layer of pulmonary surfactant. In the conducting airways, beneath the pulmonary surfactant, is the epithelial lining fluid (ELF), which blankets the predominantly ciliated epithelium interspersed with non-ciliated goblet cells and other cells. The upper layer of the ELF is a viscous mucous layer that functions to trap substances. Beneath this mucous layer is a less viscous fluid that enables the cilia to move in coordinated waves to act as a mucociliary escalator; this sweeps entrapped particles upwards to the pharynx to be swallowed. This mucociliary clearance is one of the main mechanisms of removing insoluble matter from the upper parts of the lung and is thus particularly important for slowly dissolving drug particles[5].

As the aerosol cloud moves deeper into the lungs, it faces a decreasing airway caliber, a thinning epithelium and a reduction in the fraction of ciliated cells. Furthermore, as the airways become smaller, the thick mucous layer becomes discontinuous, eventually disappearing as the respiratory bronchioles are reached. When the aerosol reaches the respiratory region, there is an exponential increase in absorptive surface area. The surface area of the airways is ~2.5 m^2, whereas the surface area of the alveolar region can exceed 100 m^2 (Ref. 6). In the alveoli, the environment for absorption is dramatically different from the conducting airways. The aerosol particle deposits on the surface lining layer (SLL), which is a dual layer comprising a thin layer of alveolar surfactant overlying an amorphous hypophase. The surfactant layer is composed primarily of phospholipids and proteins [e.g. surfactant protein A (SP-A)], and the hypophase is a fluid that contains tubular myelin. The hypophase collects in crevices between alveolar cells but on the alveolar cell surface it becomes more or less as thin as the gycocalyx[7].

Beneath the hypophase lies the alveolar epithelium, which is composed primarily of flat type I cells and cuboidal type II cells. The type I cells are very thin (<0.2 μm) with a large diameter (~200 μm; Ref. 8). Because of their large diameter, they constitute 93–97% of the alveolar epithelial surface area, even though they represent ~33% of the total number of epithelial cells. The cuboidal type II cells constitute ~5% of the alveolar epithelial surface area. They are thought to produce pulmonary surfactant and differentiate to type I cells when the epithelium is damaged. Because of the predominance of thin type I cells in the alveoli, the drug solute has a very short distance to travel between the alveolar lumen and the blood, and this favors absorption.

In addition to epithelial cells, the alveoli contain freely roaming macrophages that engulf particles, potentially digest them and slowly migrate with their payload up and out of the respiratory tract, either following along the mucociliary escalator or entering (to a lesser degree) the lymphatic system[9]. The pulmonary endocytosis represents the main mechanism of removing solid particles in the alveolar region[10]. Although generally not as important, the uptake through the lymphatic system might be relevant for macromolecules[11].

Factors affecting drug absorption in the lung

It is thought that molecules cross the pulmonary membranes by passive transport or carrier-mediated active transport. In addition, drug molecules can pass through the lung membranes via pinocytosis or bulk flow through large and small pores[12]. Early studies indicated that for substances likely to be absorbed through passive diffusion, alveolar drug absorption is faster than tracheobronchial drug absorption owing to the large alveolar surface area and the short distance between the alveolar and capillary lumens[13]. It was also demonstrated that more lipophilic and nonionized drugs were absorbed better than their more hydrophilic and ionized counterparts[13]. In addition, a relationship between molecular weight and absorption rate has been established[14].

Macromolecules are absorbed in the lungs to a degree that is, broadly speaking, inversely proportional to their molecular weight[6]. It appears that macromolecules can cross the alveolar epithelium by several mechanisms. For large macromolecules >40 kD, transcytosis may be the dominant mechanism, whereas for smaller macromolecules, both transcytosis and paracellular (between the cells) mechanisms may be important.

Methods used to assess pulmonary drug absorption

There are several methods that can be used to investigate pulmonary drug absorption. These range from *in vitro* cell culture methods, which are primarily used as screening tools, to *in vivo* pharmacokinetic analyses that provide definitive information about the fate of the inhaled drug by monitoring drug levels in plasma, lung tissue or fluid.

Cell culture methods for assessing drug transport

Both cell lines and primary cell culture methods exist for both airway and alveolar epithelia[15]. The primary cultures tend to better approximate native epithelia, whereas the cell lines provide a model that is more controllable, reproducible and sustainable.

Airway epithelial cell cultures Primary cultures of airway epithelial cells have been developed for several species including rats, guinea pigs, hamsters, rabbits and humans. Examples of the application of primary epithelial cell cultures include investigations of the influences of lipophilicity and molecular size on epithelial permeability[15].

Two human cell lines, 16HBE14o– and Calu-3 cells, can be used as absorption models[16]. The Calu-3 cell line has tight junctions; the 16HBE14o– cell line has a similar morphology to native airway epithelia, including cilia and tight junctions. These cell lines were used to show different absorption pathways for salbutamol and formoterol[17].

Alveolar epithelial cell culture Alveolar epithelial cell cultures are prepared from alveolar type II cells. Primary cell cultures that can assess drug transport include those developed from human lung[18]. An important feature of primary type II cell cultures is that they differentiate to monolayers of cells with type I cell morphology. Type II cell lines, exemplified by the human lung adenocarcinoma A549 cell line[19], form monolayers that are morphologically and biochemically distinct from native alveolar epithelia. However, cell cultures have several disadvantages[19]: some clearance mechanisms are not present in cell culture (e.g. mucociliary transport and phagocytosis), and the air–water interface and the change in permeability during breathing are difficult to mimic. They are, however, valuable tools for assessing drug transport.

Isolated lung perfusion models

Isolated lung perfusion models can be used to evaluate the fate of drugs in the lungs. Their advantages include the detailed investigation of pulmonary dissolution, absorption, lung-tissue binding, pulmonary transport phenomena and metabolism without being influenced by extra-pulmonary factors. Isolated perfused lung systems have been described in rabbits[20], rats[21,22] and guinea pigs[22].

In general, the lungs are removed from the animal and placed in a water-jacketed glass thorax. Perfusate buffer is pumped through the pulmonary artery and either collected or recirculated after leaving the lung via the venous lung outlet. Typically, the lungs are ventilated during this time. The drug can be delivered into the airways, usually by intratracheal instillation. This approach can be modified by delivering the drug via modified metered-dose inhalers[21].

Isolated perfused lungs are valuable for absorption studies[22,23]. Lung effluent (leaving the lung from the venous side) is continuously collected and analysed. These data can then be transformed into plots showing the fraction of dose absorbed over time (Fig. 1), thus permitting the determination of absorption rate and pulmonary residence time. Such systems were used for the characterization of polypeptide absorption[24] and for detailed investigations on the dissolution and absorption behavior of drugs or model compounds[25].

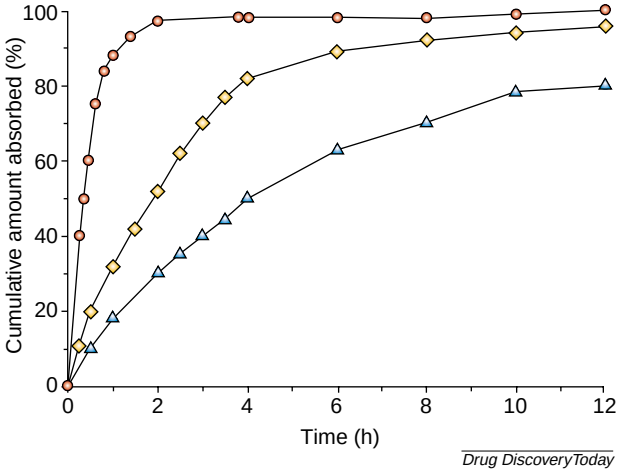

Figure 1. Possible absorption profiles obtained from experiments with the isolated perfused lung or absorption profiles obtained after proper deconvolution from inhalation studies. Lines represent drugs with different absorption rates: Circles = fast; diamonds = intermediate; and triangles = slow absorption rates, respectively.

Pharmacokinetic assessment of drugs for local activity in the respiratory tract

Rationale Traditional pharmacokinetic studies can be used to determine the degree of pulmonary deposition and the fate of the drug after inhalation. The pulmonary fate of a drug can be described in terms of residence time and pulmonary deposition[26], both of which are factors determining pulmonary selectivity. For example, simulations performed for the central part of the lung (Fig. 2) indicate that a drug with a short absorption period, when given in solution, does not exhibit pulmonary targeting because the pulmonary residence time is too short (Fig. 2a). For slowly dissolving drug particles, pulmonary targeting is not pronounced (Fig. 2c) because of mucociliary clearance of the particles. Therefore, for the upper parts of the lung, an optimal pulmonary residence time exists at which pulmonary selectivity is maximized (Figs 2b,d). Similar simulations for the peripheral part of the lung show that prolonged pulmonary residence time improves pulmonary selectivity, assuming that the anti-asthmatic effects of drugs are also mediated in this region of the lung. Thus, the assessment of the pulmonary absorption rate is an important parameter to be considered for the evaluation of an inhalation

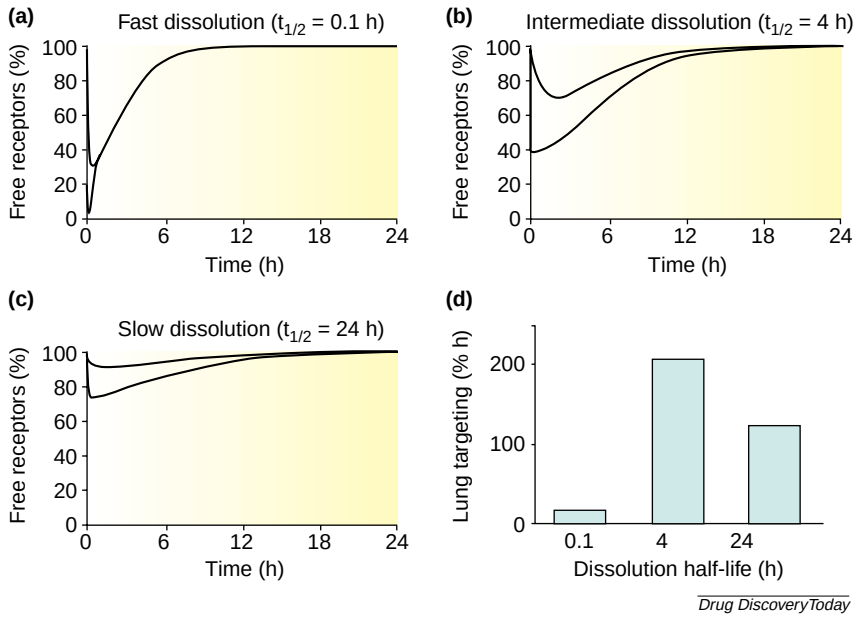

Figure 2. Simulations of the effect of the pulmonary dissolution rate on pulmonary selectivity generated by simulation using a pharmacokinetic and pharmacodynamic model describing pulmonary and systemic receptor occupancies. The inhaled dose was allowed to dissolve immediately **(a)**, with a half-life of 4 h **(b)** or with a half-life of 24 h **(c)**. Free receptors in the lung are indicated by the lower line; free receptors in the systemic circulation are shown by the top line. Pulmonary targeting is indicated when more receptors are occupied (i.e. less receptors are free) in the lung than in the systemic circulation. Pulmonary targeting (areas between pulmonary and system receptor occupancies) observed in a–c are summarized in **(d)**.

drug. Because of this, the measurement of absorption kinetics is important for assessing the bioequivalence of generic inhalation drugs, particularly when combined with knowledge of aerosol deposition sites[27] and drug effects[28].

Approaches to the pharmacokinetic assessment of pulmonary drug absorption

The pharmacokinetic assessment of the pulmonary fate of a drug can be done by a variety of approaches, including the determination of drug levels in the lung itself. Such studies have been performed in lung cancer patients who inhaled the drug before resectomy of sections of the lung. Determination of drug levels in a variety of patients at different time points enables the assessment of pulmonary kinetics. Such studies, however, show high variability and can be affected by pathological changes in the lung cancer patients. Although they are sufficient to describe the absorption profiles for drugs with slow absorption kinetics, they can only be used to describe pulmonary selectivity if the plasma and tissue levels are available after inhalation and intravenous injection[29]. Other approaches determined the pulmonary fate of drugs by analysing drug concentrations in bronchopulmonary lavage fluid. This methodology is tedious, difficult to standardize and has not been used frequently[30]. Alternative methods such as the use of microdialysis might be complementary to these methods.

The more established way of assessing the fate of a drug after inhalation has been by standard pharmacokinetic approaches using plasma–concentration time profiles. After inhalation from dry powder or metered-dose inhalers, only 10–50% of the emitted drug dose is delivered to the lung, and a substantial amount of the drug is swallowed and available for oral absorption. The plasma concentration–time profiles obtained after drug inhalation might therefore include contributions from both pulmonary and gastrointestinal absorption. Thus, for inhaled drugs, non-pulmonary absorption must be considered when evaluating pulmonary absorption using pharmacokinetic analyses of plasma samples. For drugs that exhibit negligible oral bioavailability, such as fluticasone propionate, plasma concentration–time profiles are sufficient to characterize pulmonary absorption because the drug can only enter the circulation through the lungs. For drugs with significant oral bioavailability, different approaches can be used to account for oral absorption.

The charcoal-block technique The absorption of the orally swallowed fraction of the inhaled product can be blocked with charcoal (i.e. a 'charcoal-block') in order to accurately delineate pulmonary absorption. Typically, this is done by giving concomitant doses of charcoal to adsorb the drug (Fig. 3). This approach has been used for terbutaline[31], triamcinolone acetonide[32], budesonide[33] and other glucocorticoids.

Typically, in a charcoal-block study[33], the mouth is thoroughly rinsed with a charcoal slurry, which is swallowed immediately before drug inhalation. Charcoal administration is repeated 5 min, 1 h and 2 h after drug inhalation. The charcoal-block approach was used to investigate the bioavailability of inhaled triamcinolone acetonide[32]. Using the charcoal-block technique, the researchers were able to delineate pulmonary bioavailability (10.4%) from absolute bioavailability, which was 25% of the inhaled dose. However, great care must be taken to ensure that for

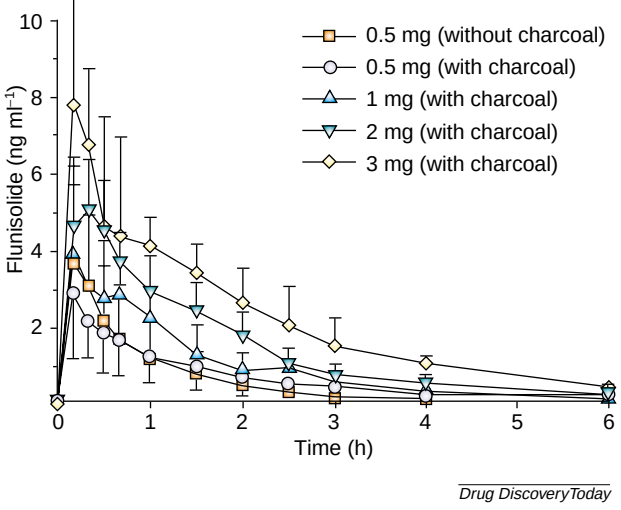

Figure 3. Plasma concentration–time profile after inhalation of different doses of inhaled flunisolide. Note the differences in AUCs between experiments with and without charcoal (0.5 mg dose). Data were taken from Ref. 34.

a given drug under the specific conditions of the clinical trial, a complete block of the oral absorption is actually observed. This might not have been the case in some of the reported studies[34].

Using different absorption kinetics to account for oral absorption The different kinetics of drug absorption from the lung and the gastrointestinal tract can also be used to assess the pulmonary absorption of inhaled drugs[35,36]. For example, after oral administration of salbutamol, the maximum urinary excretion rate is reached after 2 h; by contrast, for a lung-deposited drug, the maximum is reached after 30 min. Therefore, urine sampling up to 30 min after inhalation of salbutamol reflects the pulmonary-deposited fraction of the dose, and can therefore be used to assess the pulmonary absorption of the drug.

The existence of time lags between oral and pulmonary absorption can also be used to characterize absorption. Generally, it can be assumed that the pulmonary absorption is the faster absorption component. However, these methods are less reliable because variability in the absorption rates will significantly affect the results[37].

Pharmacokinetic parameters used to characterize pulmonary absorption Several pharmacokinetic properties can be used for the characterization of the absorption process. Typically, t_{max} (time to achieve maximal concentration) is applied to express the rate of absorption. However, t_{max} not only depends on drug absorption rate but also on the distribution and elimination rate. Drugs with multi-compartmental kinetics after intravenous administration often exhibit a low t_{max} even if pulmonary absorption is rather slow. For example, t_{max} values for fluticasone propionate have been reported to be between 0.6 h and 1.0 h (Refs 38,39), whereas budesonide, which exhibits relatively fast absorption, has a shorter t_{max} of between 0.3 h and 0.46 h (Ref. 33). For drugs that exhibit rapid absorption, such as flunisolide (Fig. 3), t_{max} often represents the first measurement point when standard sampling regimens are used, although great care must be taken to include frequent sampling at early time points to provide reliable estimates of t_{max}. In general, the use of the t_{max} value for describing the absorption kinetics is not recommended.

A more sensitive parameter of the absorption profile is the mean absorption time. In this case, the drug is given both intravenously and by inhalation. The difference between the intravenous and inhalation mean residence times will give the mean pulmonary absorption time. The advantage of this approach is the non-compartmental nature of the assessment, thus it does not depend on the correct selection of a compartmental model.

Recently, more complex pharmacokinetic tools have been used to characterize the absorption profiles by a deconvolution method[40,41]. If detailed concentration–time profiles are available after inhalation and intravenous administration, this method will generate full absorption profiles similar to those obtained from the isolated perfused lung preparations (Fig. 1). This will yield information that is not readily available from non-compartmental analysis. For example, it was determined that 50% of the lung-deposited dose of fluticasone propionate is absorbed within 2 h, whereas the remaining dose is absorbed more slowly, resulting in 90% being absorbed by 12 h (Ref. 40). An important feature of the deconvolution approach is that it enables a detailed compartmental characterization after inhalation and intravenous administration. It is anticipated that deconvolution approaches, when performed correctly, will enable one to compare, with high resolution, the absorption profiles of different formulations. Because of the importance of absorption profiles for determining pulmonary bioequivalence, such absorption studies might be more frequently used in the development of generic inhalation products.

Lung deposition

There are several techniques available to describe lung deposition, including *in vitro* approaches (the most well known being the Andersen cascade impactor), imaging approaches and pharmacokinetic studies.

Andersen cascade impactor

The Andersen cascade impactor is based on the assessment of the aerodynamic particle size using a multistage approach and is able to determine the respirable fraction of an emitted dose. Andersen cascade impactor studies represent routine studies for the evaluation of inhalation devices and provides significant information during the development stages on deposition characteristics of the formulation. More studies demonstrating *in vitro* and *in vivo* correlation need to be provided to further validate this approach as a valid tool of clinical pharmacological relevance. Multistage apparatus should be used to characterize particle size distribution, measurements should be made at flow rates reflecting those in the clinic and 'encouragement should be given to the further development, standardization, and validation of apparatus with a "throat" that more closely resembles the human oropharynx and larynx'[37].

Imaging techniques to assess pulmonary drug deposition

In vivo radiographic imaging techniques are proving to be useful in assessing drug deposition via many routes of administration[42,43]. These studies use either a radioactively labeled drug molecule or dry powder, or the MDI propellant is coated or the formulation physically mixed with a radiolabeled substance (e.g. gamma-emitting nuclide ^{99m}Tc). After inhalation, the radioactivity is monitored using a variety of scintigraphic techniques. The potential imaging techniques include planar gamma scintigraphy, single photon-emission computed tomography (SPECT), positron-emission tomography (PET) and, for limited applications, MRI, which does not require radioactive labeling.

Gamma scintigraphy Gamma scintigraphy has been used extensively in evaluating deposition patterns of inhaled aerosols[44]. Typically, the formulation is labeled with the gamma-emitting nuclide ^{99m}Tc in the form of a 'cocktail'. Immediately following inhalation of the radiolabeled aerosol, a gamma camera is used to record images and to obtain radioactive counts of the whole lung, lung zones (central, intermediate and peripheral), oropharynx, esophagus and stomach. The resulting images of such experiments provide an immediate impression of where the drug is deposited, provided, of course, that the label follows the drug with high fidelity. Whole lung deposition and regional deposition can be calculated as a fraction of total dose or other relevant parameter (e.g. peripheral-to-central deposition ratio). Results from these deposition studies can be a useful adjunct for comparing the efficiency of delivery devices and for evaluating the equivalence of inhalation products. Conventional planar gamma cameras can only generate a two-dimensional picture of the body and it is not possible to distinguish between the conducting airways and alveolated parts of the lung.

Using the marker technetium-99m-labeled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA), gamma scintigraphy has been used extensively to measure epithelial permeability in the lungs[45]. ^{99m}Tc-DTPA is a small (MW = 492 Da) hydrophilic compound that is administered by instillation or aerosolization into the lungs. Following a time sequence of gamma camera photographic images, the clearance of radiolabeled solute can be calculated. However, there are several important considerations for using this marker[46].

Gamma scintigraphy is also beginning to find utility for examining the clearance of ^{99m}Tc-labeled drugs. For example, gamma scintigraphic studies suggested that intratracheally instilled ^{99m}Tc-tobramycin was predominantly cleared from the lung via systemic absorption[47]. Further, using this method, mucociliary clearance could account for a significant fraction of ^{99m}Tc-DTPA clearance in the lungs[48]. This technique was also used to further our understanding of pulmonary deposition and the fate of proteins and peptides in the 1990s (Refs 49,50).

One limitation of conventional gamma scintigraphy is that it can only generate a two-dimensional picture of the body and it is not possible to distinguish between certain overlying structures. In addition, the labeling procedure involves manipulations of the formulations. Consequently, the formulation labeled might be different from the formulation intended to be tested. Agencies such as the FDA are therefore very cautious in using results from imaging studies for assessing bioequivalence.

Other imaging techniques In addition to gamma scintigraphy, single photon-emission computed tomography (SPECT) and positron-emission tomography (PET) has been used to examine the fate of inhaled drugs[51–55]. An advantage is that these methods can be used to construct three-dimensional images. Compared with planar gamma scintigraphy, PET and SPECT enable the characterization of pulmonary deposition with higher resolution and the obtainment of a better distinction between central and peripheral lung deposition or pulmonary and gastrointestinal deposition. An additional advantage of PET is that it enables the drug to be labeled itself without modifying its chemical structure and can thereby overcome some of the limitations of scintigraphy. Labels used in PET include ^{11}C, ^{13}N, ^{15}O and ^{19}F.

Pharmacokinetic assessment of pulmonary bioavailability

The fraction of an inhaled drug absorbed through the lung can be obtained from concentration–time profiles

by comparing the area under the concentration–time curve after inhalation (AUC_{inh}) with that observed after intravenous administration (AUC_{iv}). In the case of drugs with negligible oral bioavailability, the direct comparison will yield the pulmonary bioavailability of the inhaled drug.

For drugs with significant oral bioavailability, the AUC_{inh} obtained without charcoal blocking ($AUC_{inh,noChar}$) can be used to calculate the overall bioavailability of the inhaled drug; however, this will include oral (gastrointestinal) bioavailability. The pulmonary bioavailability of the inhaled drug can be calculated from a comparison of $AUC_{inh,noChar}$ with AUC_{inh} plus efficient charcoal blocking ($AUC_{inh,Char}$). When inhaled and intravenous doses are identical, the oral bioavailability (F_{oral}) and the pulmonary bioavailability (F_{pulm}) of the inhaled drug can then be derived from Eqns 1,2.

$$F_{oral} = (AUC_{inh,noChar} - AUC_{inh,Char})/AUC_{iv} \quad [1]$$

$$F_{inh} = AUC_{inh,Char}/AUC_{iv} \quad [2]$$

Characterization techniques similar to the ones described above have been used for several inhalation drugs. These studies demonstrated that pulmonary bioavailability differs among formulations used and often ranges from 10% for older metered-dose inhalers (MDIs) to 30–50% for newer MDIs and dry powder inhalers (DPIs). Differences in the systemic availability between devices such as MDIs and DPIs were described using pharmacokinetic approaches[56]. Studies comparing MDIs and DPIs found that lung deposition also differs between these devices[33]. In addition, differences in the systemic availability between chlorofluorocarbon and hydrofluoroalkane MDI formulations have been found for formulations of beclomethasone dipropionate[57]. Pharmacokinetic approaches were also used to demonstrate differences in the pulmonary deposition and systemic absorption of fluticasone propionate between healthy volunteers and asthmatics[58].

The approach of determining the systemic availability of an inhaled drug will also be very useful in bioequivalence studies because this approach will enable the comparison of the systemic exposure of two formulations, as well as the amount of drug deposited in the lung, when studies are performed with sufficient charcoal block. The simplicity and lack of controversy of performing these pharmacokinetic studies compared with other methods, such as scintigraphy, indicates that pharmacokinetic studies will be more widely used in the future for the assessment of pulmonary bioequivalence[59].

Pharmacodynamic assessment of pulmonary targeting

Pulmonary effects as well as the ratio of pulmonary effects and systemic side effects have been used to assess pulmonary targeting.

Lung targeting in rats Different pharmacodynamic approaches have been used to assess pulmonary targeting in rats. Instillation of Sephadex into the lungs of rats induces pulmonary inflammation, which results in edema and weight gain of the lungs[60]. It is possible to measure the effects of glucocorticoids by measuring the inhibition of pulmonary weight gain. Pulmonary targeting can be assessed by locally treating one lobe and measuring the effects on both lobes. Pulmonary targeting is then observed if only a reduction in lung weight is observed in the locally treated lobe. Using this system of alveolar inflammation, distinct pulmonary targeting was observed for budesonide-21-palmitate liposomes, whereas no targeting was observed with budesonide[60].

Further, in rats, glucocorticoid receptor occupancies in the lung and the systemic circulation have been examined in order to assess pulmonary targeting[61]. In these experiments, the drug is given intratracheally to rats and the amount of free receptors are determined in the lung and the liver.

Pharmacodynamic assessment in humans Pharmacodynamic studies of pulmonary targeting have also been performed in humans. For example, budesonide was administered either orally or through inhalation to obtain similar systemic drug levels[62]. The pulmonary effects and the effects on systemic surrogate markers were determined. More pronounced pulmonary effects observed after inhalation indicated pulmonary targeting.

Pharmacokinetic (PK) and pharmacodynamic (PD) correlations were used to assess the pulmonary targeting of inhaled β-2-adrenergic drugs[63]. A PK and PD analysis of the pulmonary effects after systemic administration defined the relationship between plasma levels and pulmonary effects, and enabled the prediction of pulmonary and systemic effects from plasma levels after intravenous administration. If a drug is inhaled and no pulmonary targeting is observed (i.e. free levels in lung are identical to free levels in the systemic circulation), the plasma levels of the drug should also describe its pulmonary effects. If the free drug levels after inhalation are higher in the lung than in the systemic circulation, pulmonary effects predicted from plasma levels will be smaller than the clinically observed effects on airway resistance but should still describe the systemic side-effects. The difference between observed and predicted values indicate the degree of pulmonary targeting (Fig. 4).

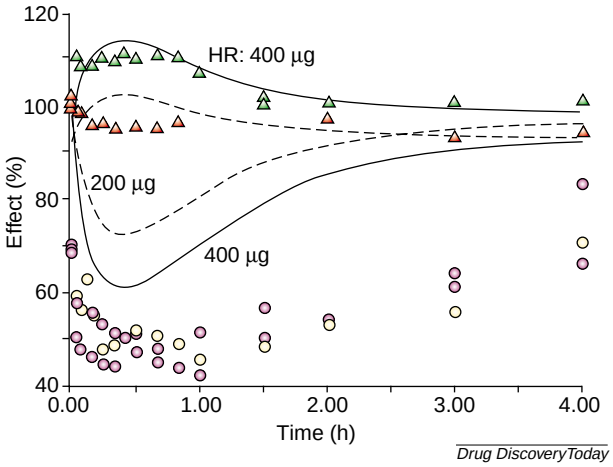

Figure 4. Effects on heart rate (triangles) and on reduction in pulmonary resistance (circles) observed after 200 µg (closed symbols) and 400 µg (open symbols) of inhaled fenoterol. Although plasma levels are a good descriptor for systemic effects (HR), plasma levels cannot be used to predict effect on the pulmonary resistance. The difference between the lines and the datapoints represents pulmonary targeting. Data were taken from Ref. 65.

Similar approaches to the characterization of systemic and pulmonary effects after inhalation have been to used to determine the bioequivalence of β-2-adrenergic drugs[28,64]. Based on the assessment of pharmacodynamic endpoints, guidances have also been published, for example, by the Canadian authorities (http://www.hc-sc.gc.ca/hpb–dgps/therapeut/zfiles/english/guides/mdi/mdiatt_e.html).

Conclusion

There are several methods that can provide specific information on the pulmonary disposition of drugs. Each of these methods provides a 'puzzle piece' in describing the pulmonary fate of inhaled drugs and, in combination, they will provide a relatively complete picture of the performance of the pulmonary drug delivery device. With pulmonary delivery gaining more and more importance, methods used to characterize pulmonary deposition and absorption may improve within the next decade. New approaches such as microdialysis will be used, improved scintigraphic methods will be developed and a more detailed understanding of the relationships between regional deposition and pharmacological effects will be generated.

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