In Vivo Measurements of Aerosol Dose and Distribution: Clinical Relevance

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ABSTRACT

Mathematical and in vitro models, that incorporate particle diameter, normal breathing frequencies and tidal volumes, have been used to predict the deposition fraction of respirable aerosols within the lungs. Although very useful in drug development, determinations of dose and the distribution of dose based solely on such models may be less accurate than in vivo measurements, which are performed under conditions that combine the effects of all the factors that determine aerosol deposition, including the effect of disease. Gamma scintigraphy provides a method for in vivo quantification of the total deposited fraction and the distribution of the dose within the lower respiratory tract. Using this technology, it has been shown that deposition fraction in the lower respiratory tract may vary between 1-30% of the dose actuated from an MDI or nebulizer. This wide range in deposited dose suggests that variations in the clinical response to inhaled aerosols may be explained by alterations in the dose delivered, especially if the aerosolized medication has a narrow therapeutic range. Alterations in the distribution of inhaled drugs within the lungs may also affect the clinical response, such that some disorders may best be treated by targeting drug to specific locations of the lung, while others may respond better to homogenous distribution of aerosolized drug. In vivo measurements would provide confirmation of the dose deposited as well as the pattern of distribution, which should improve the therapeutic outcome of most aerosolized medications.

INTRODUCTION

Aerosol medications are replacing orally administered and intravenously injected drugs in the treatment of respiratory diseases and may come to play a major future role in the delivery of some drugs into the systemic circulation. In treating respiratory diseases, aerosol therapy often requires a much smaller dose of drug to achieve the desired response, produces a more rapid onset of action and results in a lower incidence of systemic side effects. Medications currently delivered as aerosols to the lungs as target organ include: bronchodilators that reverse bronchospasm; bronchoconstrictors that are used to diagnose airway hyperresponsiveness; steroidal and non-steroidal agents for the treatment of inflammation; antibiotics for the treatment of infection in cystic fibrosis (CF) patients and for the prevention of infection in HIV-positive patients; and anti-viral drugs that are administered to children with respiratory syncytial virus. One category of drug that is actively being considered for aerosolized delivery as a means of entry into the systemic circulation is peptides. Success with these would undoubtedly stimulate work on other possible categories.

Key Words: aerosol deposition, aerosol therapy, gamma camera imaging, human respiratory tract
With the lungs as the target organ or serving as an alternative route of administration for drug delivery into the systemic circulation, an adequate dose of medication must be deposited beyond the oropharynx to achieve a full therapeutic effect. In addition, the distribution of drug within the lungs (i.e., uniform vs. non-uniform, central airway vs. peripheral airway) may also play a role in optimizing the effect of therapy. To maximize aerosol delivery to the lungs and at the same time target specific regions for deposition, determinants of aerosol deposition must be understood, and wherever possible, regulated. Factors that influence the dose and the distribution of the dose of aerosol in the human respiratory tract include: aerosol particle size characteristics, aerosol velocity, inspiratory flow rate, breathholding time, lung volume and airway calibre. Mathematical and in vitro models that incorporate many of these factors have been used to predict the deposition fraction of inhaled aerosols within the lungs. Although useful in drug development, determinations of dose and the distribution of dose based solely on such models may be less accurate than in vivo measurements, which are performed under conditions that combine the effects of all the factors, including the effect of disease. 

Gammascintigraphy provides a method for in vivo confirmation of the dose deposited as well as the pattern of distribution. Gammascintigraphic assessment should therefore be useful in improving therapeutic outcomes for many aerosolized medications. Methods for quantifying the total amount and distribution of delivered dose and the advantages and disadvantages in using gammascintigraphy are discussed by Gonda, et al. in these proceedings and have been reviewed by Cross, et al. (1992) and Newman, et al. (Moren, Dolovich, Newhouse and Newman, 1993) elsewhere.

**IN VIVO MEASUREMENTS OF DOSE: CLINICAL RELEVANCE**

**Particle Size**

The Task-group on Lung Dynamics developed a model that has been a useful predictor of deposition fraction in the lungs as a function of aerosol particle size characteristics (Task-group on Lung Dynamics, 1966). This model predicts that, during inhalation, the majority of particles >5 μm in aerodynamic diameter will impact and be retained in the upper airways (nasopharynx or oropharynx). Particles <5 μm in diameter will penetrate beyond the upper airways and deposit in the tracheobronchial and pulmonary regions of the lungs. Particles <5 μm are, therefore, called respirable. These predictions have been confirmed in vivo using gamma camera imaging technology (Newman et al., 1988). Data from that study are shown in Figure 1. In those experiments, seven

![Image of gamma camera scans of aerosol deposited in the posterior lungs (L), oropharynx (O), and stomach (S) of a CF patient after inhaling a radiolabeled comprised of 7.3 μm or 3.2 μm particles. Reprinted by permission from Ref. (Newman et al., 1988).](attachment:image.png)
patients with CF inhaled the antibiotic carbenicillin, radiolabeled with technetium-99m. Radioaerosol was generated by two different nebulizer/compressors, producing aerosols with mass median diameters of 3.2 or 7.3 μm. Figure 1 shows the gamma camera images of the lungs of one of the CF patients in the study after inhaling the two radioaerosols. Contour lines represent lung outlines (10% and 30% of maximum count rate, respectively) as defined with inhaled radioactive gas. As predicted by the Task-group model, dose deposited within the lungs was greatest for the 3.2 μm aerosol, whereas a larger fraction of the 7.3 μm aerosol was retained in the oropharynx and was swallowed (stomach activity). In addition, these in vivo measurements demonstrated that the distribution of aerosol within the lungs was significantly more peripheral with the smaller size particle.

Rees et al. (1982) related particle size to the therapeutic effect of the bronchodilator terbutaline sulphate, in ten asthma patients. Patients inhaled terbutaline aerosol, generated by a metered dose inhaler (MDI), consisting of <5 μm particles, 5-10 μm particles, or 10-15 μm particles on three different occasions. Measurements of airways resistance and flow-volume were obtained before and immediately after aerosol inhalation. Changes in specific conductance (SGaw), the forced expiratory volume in one second (FEV1) and flow at 50% vital capacity (V50) were expressed as an increase from baseline over 60 minutes. Their findings are summarized in Figure 2. The greatest increases in the three measurements were produced by the aerosol with the smallest particle size. Thus, all three measurements were significantly increased at each of the time-points by the <5 μm particles. The authors concluded that these results were due to a larger dose of bronchodilator deposited within the lungs when patients inhaled the smaller aerosol particles.

Within the respirable category, the coarser particles provide a larger dose of drug than the finer particles because of differences in their mass and deposition efficiency. Differences in dose can be overcome by increasing the concentration of drug for the finer particles. Figure 3 shows the results of a study by Dolovich et al. (1992) where equal doses of a bronchodilator were delivered to the lung by aerosols consisting of 0.5 μm or 2.4 μm particles. They found that when the cumulative dose (micrograms) to the lungs was matched, percent change in pulmonary function, as measured by FEV1, was the same for the two aerosols.

**Aerosol Velocity**

There are a number of other determinants of the dose of inhaled medications deposited within the human respiratory tract. Thus, impaction of aerosol particles within the oropharynx and the concomitant loss of drug not only occurs because of large particle size but also because of high aerosol velocity (Laubc et al. 1984). For example, the velocity of an aerosol spray as it leaves a pressurized canister may exceed 50 m/sec (Rance, 1974). Using a 2-crystal shadow shield whole-
body counter in a scanning (profile) mode, Newman et al. (1981) quantified the fraction of radiolabeled, monodisperse, teflon particles (3 μm) deposited in the mouth, lungs and stomach of eight patients with chronic obstructive airways disease, who inhaled the aerosol from a pressurized canister. He also obtained gamma camera images to give a pictorial view. Figure 4 is a typical composite view of the head, lungs and stomach of one of the patients in the study. Deposition within these regions was expressed as a percentage of the inhaled fraction. They found that only 8.8% of the inhaled dose deposited in the lungs, 5.8% depositing in the conducting airways and 3% in the alveoli. Approximately 80% was lost in the mouth due to inertial impaction. These high losses were due to a combination of the large effective particle size (incomplete evaporation of the propellants that coat the particles leads to an increase in particle size) and the high aerosol velocity attributed to these particles as they left the canister.

**Inspiratory Flow Rate**

Inspiratory flow rate also affects the dose of aerosolized medications deposited in the lungs (Newhouse et al., 1986; Newman et al., 1983; Newman et al., 1982; Lippmann et al., 1969; Pavia et al., 1977; Laube et al., 1984). This is illustrated in Figure 5. Three normal subjects inhaled a radiolabeled aerosol, generated by nebulizer, at increasing inspiratory flow rates. Single probe gamma detectors collimated to record counts only from the bronchopulmonary region (below the larynx) or the oropharynx were used to quantify the total deposition fraction. Counts within the bronchopulmonary region were expressed as a percentage of total deposition. Between 13 and 35 L/min, fractional deposition in the lungs was reproducibly maximized in these three subjects and ranged from 70 to 90%. Above 35 to 40 L/min, there was a dramatic decrease in the bronchopulmonary deposition, indicating that oropharyngeal deposition had increased.

Newman et al. (1980) examined the effect of inspiratory flow rate on the pulmonary response to an inhaled bronchodilator aerosol. Results from that study are summarized in Figure 6. When asthma patients inhaled the bronchodilator terbutaline sulphate at a slow inspiratory flow rate of approximately 30 L/min, the percentage increase in FEV₁ was significantly greater for all times post-inhalation than when the drug was inhaled at a faster rate of approximately 80 L/min. Faster inhalation was less effective because more drug impacted in the oropharynx and was lost.

FIGURE 5. The Effect of Inspiratory Flow Rate on Bronchopulmonary Deposition in Three Normal Subjects (crosses, subject 1; shaded circles, subject 2; filled circles, subject 3). Reprinted by permission from Ref. (Laube et al., 1984).
**Breathholding Time and Lung Volume**

Particles > 0.5 μm that penetrate to the smaller airways deposit by sedimentation as the result of gravity. Breathholding for up to ten seconds enhances the residence time and so increases deposition by sedimentation (Palmez, 1973). When inspiratory flow rate at the time of inhalation is slow (approximately 30 L/min) and the breathholding time is for ten seconds, lung volume at the time of inhalation does not appear to be critical to the dose deposited within the lungs (Newman et al., 1982). However, since airway size varies with lung inflation, the time for sedimentation is important at higher lung volumes because distances for settling are greater. Thus, when the breathholding time is only four seconds, lung volume at the time of inhalation may significantly affect the dose deposited (Newman et al., 1982). Figure 7 illustrates the effect of breathholding time and lung volume on aerosol deposition. For Group B patients (ten second breathhold), whole lung deposition of radiolabeled teflon particles was similar for the three lung volumes tested. In Group A patients (four second breathhold), deposition in the whole lung was similar to Group B patients when lung volume at the time of inhalation was 20% of the vital capacity, but not when lung volume was 50% or 80% of the vital capacity. Newman et al. (Baran, 1979) also found that improvement in pulmonary function following inhalation of a bronchodilator, measured in terms of percent change in FEV₁, was similarly associated with breathholding time and lung volume.

Over the years, a number of investigations have examined aerosol dose to the lungs with various nebulizer and MDI delivery systems (Davies, 1975, Spiro et al., 1984, Short et al., 1981, Newman et al., 1981, Dolovich et al., 1981, Davies, 1982, Asmundsson et al., 1973, Bau et al., 1971, Ruffin et al., 1978, Lewis et al., 1981, Gottschalk, et al., 1978, Wasnich, 1976, Lin et al., 1974). Although the deposition fraction for these investigations averages 10-15%, estimates vary from one or two percent to 25 to 30% between studies and between patients in the studies. This variability may be the result of differences in the particle size of the aerosol generated by the nebulizers or MDIs, may be due to differences in the breathing maneuvers of the patients during inhalation, or may be the result of differences in the disease state of the patient population. Variations in dose deposited in the lungs could significantly impact the treatment outcomes of many aerosolized drugs, especially those with a narrow therapeutic range. An example of a medication with a narrow therapeutic range is insulin. Previous studies in animals and humans demonstrated the bioavailability of insulin delivered to the systemic circulation by oral inhalation, since plasma insulin increased after inhalation (Cresia et al.,

**FIGURE 6**. The Effect of Inspiratory Flow Rate on the Pulmonary Response to an Inhaled Bronchodilator Aerosol. Reprinted by permission from Ref. (Newman et al., 1980).
1988, Almer et al., 1988, Wigley et al., 1971, Elliott et al., 1987). These same studies showed that insulin delivered in this manner was bioactive, since plasma glucose levels decreased after inhalation. Nevertheless, only one of the patients in the two human studies combined achieved a normal plasma glucose level after inhaling insulin aerosol. The authors suggested that the reason for this poor response could be ineffective absorption of insulin across the respiratory mucosa. However, the dose of insulin that was actually deposited in the lungs after inhalation was never quantified in either study.

In the one study, the authors assumed that the amount of insulin placed in the nebulizer was aerosolized completely and delivered to the lungs (Wigley et al., 1971). However, it is likely that many of the aerosol particles were lost as a result of impaction on the nebulizer walls or in the oropharynx. In the other study (Kelliher et al., 1987), the authors assumed that each dose that was actuated by the nebulizer, quantified by weighing the nebulizer before and after actuation, was delivered to the lungs of the patients. Yet, most of the aerosol was probably lost by impaction in the nebulizer or in the spacer and tubing attached to the nebulizer. Thus, an inadequate dose of insulin deposited in the lungs could also explain the poor response to inhaled insulin reported in the previous studies. We repeated these earlier experiments, but were careful to quantify the dose that was available for inhalation at the mouth from the delivery system as well as the dose that was actually deposited in the lungs (Laube, et al., 1993). We also manipulated the factors known to affect aerosol deposition in the human respiratory tract so as to maximize delivery of insulin to the lungs of six patients with non-insulin dependent diabetes mellitus (NIDDM), during a fasting state. For our insulin generator, we chose a nebulizer that produced particles that were small enough (mean median aerodynamic diameter = 1.12 μm) to penetrate beyond the oropharynx and deposit in the lung periphery. Losses in the oropharynx were further reduced by minimizing aerosol velocity at the mouth. This was accomplished by placing a spacer device between the nebulizer and the patients' mouth. Inspiratory flow rate at the time of inhalation was regulated at <30 L/min to reduce impaction of particles in the oropharynx and the larger, central airways. Inhalation began from residual volume to promote aerosol distribution to the lung periphery. Under these conditions of aerosol generation and inhalation, gamma camera scans of the lungs of the NIDDM patients following aerosol inhalation indicated that deposition within the lungs averaged 79 ± 17% of the inhaled dose. Figure 8 shows the time-response curves for the plasma glucose and insulin levels of one of the patients in the study, after inhaling approximately 1.0 U/kg body weight of insulin aerosol, generated and delivered as described above. In this patient,
plasma glucose levels decreased steadily to within normal values (75-120 mg/dl) over 160 minutes. Plasma insulin levels peaked at approximately 10 minutes and then began to decrease over time. Plasma glucose levels for all six patients decreased an average of 55% from baseline, and were normalized in five of the six patients. The glucose level was almost normal in the sixth patient. These findings indicate that when an adequate dose of insulin is delivered to the lungs, normalization of plasma glucose levels in NIDDM patients, during a fasting period, is feasible.

It is well-known that the effectiveness of aerosolized medications varies between patients; some being much improved, while others fail to improve. Variations in dose deposited within the lungs could account for these treatment failures. In vivo measurements would provide a method for quantifying the fraction of aerosolized medication deposited within the lungs. If the dose appeared inadequate, adjustments could be made to the delivery system, breathing maneuvers, or drug concentration to increase the dose deposited and, thereby, optimize a successful treatment outcome.

**IN VIVO MEASUREMENTS OF DISTRIBUTION: CLINICAL RELEVANCE**

**Airway Patency**

In patients with asthma, cystic fibrosis and chronic obstructive pulmonary disease, the diameter of the airways may become narrowed as the result of bronchoconstriction, edema or increased mucus secretion. These narrowed airways may then act as a filter of inhaled medications, extracting aerosol particles by impaction mechanisms and preventing distribution of drug into the lung periphery. Figures 9A and 9B illustrate this effect of airway narrowing on the distribution of aerosol particles within the lungs. In Figure 9A, aerosol deposition appears evenly distributed throughout the lungs of this asthma patient, who had a normal FEV1 (83% of predicted values) at the time of inhalation. In contrast, deposition within the lungs of the patient shown in Figure 9B, who was severely obstructed at the time of aerosol inhalation (FEV1 = 36% of predicted values), appears to be predominantly in the larger conducting airways, with very little penetration of aerosol to the lung periphery.

Newhouse et al. (1978) showed that alterations in aerosol distribution affect the pulmonary response to inhaled drugs. In that study, they preferentially deposited histamine aerosol in the larger, central airways or diffusely throughout the lungs of five asthma patients by varying aerosol particle size (i.e., 3 μm vs. 1.5 μm) and the breathing maneuver (i.e., rapid tidal breathing vs. slow deep breathing from functional residual capacity). The two deposition patterns were confirmed by analysis of gamma camera scans of the lungs following inhalation of radiolabeled aerosol. The investigators found that when histamine aerosol was selectively deposited in the central airways, less drug was...
FIGURES 9A and 9B. The Effect of Airway Narrowing on Aerosol Distribution within the Lungs. Reprinted by permission from Ref. (Laube et al., 1986).

necessary to produce a 20% fall in the FEV1 PD20 than when the drug was deposited evenly throughout the lungs.

Figure 10 shows the dose response curves for one of the patients in the study after inhaling histamine aerosol that deposited in the central airways or was diffusely distributed. The ratio for the two deposition patterns was 14:1. With central deposition, the average dose of histamine necessary to

\[ \text{Dose Ratio} = 14:1 \]

\[ \text{mg HISTAMINE IN TOTAL RIGHT LUNG} \]

FIGURE 10. Dose Response Curves for Asthma Patient Inhaling Histamine Aerosol that Deposited in the Central Airways or was Diffusely Distributed. Reprinted by permission from Ref. (Newhouse et al., 1978).
produce a PD_{20} was 35 μg for all five subjects. When deposited diffusely, an average of 494 μg of histamine was required to produce a PD_{20}.

The authors suggest that one explanation for the observed differences in the pulmonary response with the two aerosol deposition patterns may involve a greater number of histamine receptors in the larger airways than in peripheral airways. Another explanation could be that the index FEV_{1} is much more sensitive to larger airway constriction than small airway constriction. A third explanation may involve a difference in the dose per lung surface area. Calculations based on airway dimensions suggest that changing the deposition of a drug from homogeneous throughout the lungs to focal within bronchial generations 1-4 would result in a 3000-4000-fold increase in the initial airway surface concentration in these airway generations. This change should be of sufficient magnitude to influence derived indices of bronchial reactivity significantly.

We repeated the experiments of Newhouse et al. (1978) using methacholine aerosol instead of histamine in nine asthma patients (Laube et al., 1992). Distribution of the methacholine was varied by alterations in inspiratory flow rate at the time of inhalation. Quantification of changes in the pattern of distribution of methacholine aerosol was obtained from gamma camera images of the lungs following inhalation of a technetium-99m radiolabeled aerosol (no methacholine). The radiotracers were used as a surrogate for methacholine aerosol because the methacholine and radiotracers were generated by the same nebulizer and administered to the patients by the same delivery system. In addition, particle size determinations with a cascade impactor indicated that the mass median aerodynamic diameter of the two aerosols was similar. Figure 11A and 11B show gamma camera scans of the lungs of one of the patients in that study following rapid (~ 60 L/min) vs. slow (~ 12 L/min) inhalation of radiolabeled aerosol. Aerosol deposition appears most uniformly distributed, with greater penetration to the lung periphery, following the slow inspiratory maneuver. The faster inspiratory maneuver resulted in a more central deposition pattern with hot spots of focal deposition.

Frequency distribution histograms were constructed from the radiotracers images, using computer programs we developed. Distribution uniformity was quantified in terms of skew (a measure of histogram symmetry). Higher values of skew indicated heterogeneity in counts per picture element in the image and in the aerosol deposition pattern. Mean skew values were significantly higher (1.12 ± 0.35) following the rapid inspiratory maneuver compared to the slow inhalation (0.74 ± 0.36). A Spearman rank-correlation test demonstrated that values of skew were inversely correlated with the doses of methacholine that resulted in a PD_{20} (Figure 12). These studies extend the work of Newhouse et al. (1978) and indicate that shifts in distribution homogeneity are a determinant of PD_{20} during bronchoprovocation testing. These findings have important implications for standardizing bronchoprovocation challenge procedures as well as for study designs that involve comparisons of challenge results pre- and post-aerosolized drug treatment.
Martinen et al. (1991) has suggested that there may be clinical applications for preferential distribution of aerosol medications in the human respiratory tract. Specifically, he indicates that the treatment of lung diseases that manifest at well-defined locations within the lungs (i.e. bronchial carcinomas that have a predilection for developing in large airway bifurcations) may be improved by targeting aerosolized medication to that region. Similarly, targeting medication to certain receptor populations, such as respiratory vagal afferents located at airway junctures, might improve the treatment of asthma.

Anti-inflammatory drugs are thought to be most effective when deposited in the smaller airways, while the alveolar portion of the lungs may be the best target for aerosolized delivery of peptides like insulin that are to be absorbed into the systemic circulation. Maximizing the therapeutic effect of these aerosolized medications may require manipulating the factors that affect deposition so as to promote delivery to the lung periphery. Unfortunately, our knowledge of where exactly we want to target is not as advanced as we would wish, nor our ability to deliver exactly on target.

Other lung diseases might benefit from a more even distribution of aerosolized medication within the lungs. Homogeneous aerosol distribution within the lungs might improve the therapeutic efficacy of antibiotic aerosols used to treat Pseudomonas aeruginosa infections in CF patients and of anti-viral aerosols such as ribavirin used to treat bronchiolitis in infants and children. Another lung disease that might benefit from evenly distributed medication is pneumocystis pneumonia (PCP) which afflicts HIV-positive patients. Pentamidine isethionate has been shown to have some success in preventing PCP in these patients when delivered as an aerosol in a 300 mg monthly dose. However, there have been reports of patients who have developed PCP while undergoing treatment with aerosolized pentamidine. Some have observed that PCP occurs in the apical zones of these patients (Abd et al., 1988, Lowery et al., 1988). One explanation of this observation may involve an inadequate distribution of pentamidine to the apical regions of the lungs. Figure 13 shows the gamma camera images of the lungs of an HIV-positive patient in a study by Baskin et al. (1990), who investigated the effect of alterations in body position during inhalation of radiolabeled pentamidine aerosol. Pentamidine was inhaled in a sitting position starting from functional residual capacity (FRC), in a sitting position with an abdominal binder starting from FRC, in a sitting position breathing from residual capacity, and in the supine position breathing from FRC. They found that when aerosol was inhaled in the supine position, distribution of the drug was more uniform and there was improved deposition in the lung apices. It remains to be seen if changes in pentamidine aerosol distribution improve prophylaxis against PCP.
CONCLUSIONS

As the benefits of delivering medications by the aerosol route become better understood, it is important for all who are involved in new drug development, including the drug manufacturer, the regulatory agency, and the academic scientist, to be concerned with quantification of the dose and distribution of the dose of an aerosolized drug within the human respiratory tract. This is because it is likely that a successful therapeutic outcome for most aerosolized medications depends, in part, on optimizing the dose deposited and on appropriate targeting of the lung as a whole or on a regional basis. Thus, treatment failures and variations in the clinical response between patients may be explained by alterations in the dose delivered, especially if the aerosolized medication has a narrow therapeutic range. Alterations in the distribution of inhaled drugs within the lungs may also affect the clinical response, such that some disorders may best be treated by targeting drug to specific locations of the lung, while others may respond best to homogeneous distribution of aerosolized drug. In vivo measurements would provide direct confirmation of the dose deposited as well as the pattern of distribution, since they are performed under conditions that combine the effects of all the factors that determine aerosol deposition, including the effect of disease.

REFERENCES


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