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The impact of intratracheal aerosol technologies on preclinical pulmonary research

Greater control over speed, dose volume and quantification of delivered dose to the lungs enable researchers to explore new questions.

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Inhaled therapies have been documented at least as far back as ancient Egypt when medicinal herbs were burned or vaporized in public baths. Only relatively recently, however, have scientists possessed the tools to analyze the physiological, pharmacological and technological factors that determine the body’s response to inhaled drugs. The use of these tools in preclinical studies in particular has heightened our awareness of the potential benefits of pulmonary drug delivery, as well as the difficulties in achieving those benefits.

The past 25 years have witnessed a rapid expansion of the respiratory pharmacopoeia to include both liquids and dry powders in formulations from nanoparticles to macromolecules. New inhalation technologies have improved treatment and patient compliance. However, in the face of growing regulatory demands for safety and efficacy data, scientists continue to need more accurate preclinical proxies of disease, drug response and delivery technologies. This article reviews how researchers have used preclinical pulmonary drug delivery methods, in particular, intratracheal aerosol technologies, to make in vivo, ex vivo and in vitro pulmonary research more clinically relevant, effective and innovative.

Drug delivery to the lung: Who’s in charge?

The lungs offer a desirable target for prevention and treatment not only of pulmonary diseases, but systemic ones. Taking full advantage of this is challenging anatomically and technologically. In both humans and animals, the lungs are protected by anatomical barriers and defended by clearance mechanisms that can only be circumvented by particles in a size range of 1-5 µm in order to minimize impaction in the respiratory tract and achieve uniform distribution throughout the lung.

However, particle size alone does not equate to drug efficacy, safety or clinical relevance in preclinical models of drug delivery. Of equal importance is how well a given preclinical drug delivery technology lends itself to: 1) precise quantification of both the emitted and delivered dose, 2) rapid
administration of a wide range of dose volumes, 3) determination of a clearly delineated dose response curve, 4) optimal concentration, uniformity and depth of drug deposition in the lungs and 5) the ability to aerosolize a wide range of formulations from nano- to macro-scale without compromising integrity or viability.

In preclinical use, finding a drug delivery technology that offers control of all of these factors has not been easy. Inhaled drug delivery, whether in humans or animals, is inherently subject-dependent because it occurs breath by breath. As a result, the pulmonary researcher has far more control over the dose emitted by the device than over the timing or fate of the delivered dose in the lung.

With more than 80% of preclinical research being conducted in small animals, a reliance on rodents for pulmonary drug delivery has important implications.4 Mice and rats are obligate nose breathers that do not inhale orally as humans do. Major dissimilarities also exist between humans and experimental animals in the key structures of the nose and lungs, and in their relative susceptibility to disease and response to therapeutic agents.5,6 Investigators have had to take these disparities into account when developing preclinical in vivo models of disease and drug delivery for orally inhaled products.

**Initial models of preclinical pulmonary drug delivery**

Prior to the 1990s, commercially available approaches for modeling pulmonary drug delivery in the preclinical setting centered on two main approaches:

1) Liquid bolus instillation (intratracheal or intranasal). A large liquid droplet is administered to the nares or dripped down the trachea of an anesthetized animal using a syringe, pipette, catheter, gavage needle or blunt dosing needle.

2) Inhalation (intranasal or oral). A range of technologies adapted from jet, vibrating mesh or ultrasonic nebulizers or inhalers are used to emit aerosols that are typically directed at restrained or unrestrained, conscious animals housed in whole-body, head-only or nose-only exposure chambers, or are respired via an oropharyngeal airway mask adapted for larger mammals, including non-human primates.

Each of these methods offers advantages and disadvantages. Liquid bolus instillation places greater control in the hands of the user than inhalation methods, permitting rapid delivery, more precise dosing and the ability to administer far larger volumes. But it is not a model of aerosol delivery. Large droplets at the mercy of gravity typically result in uneven or patchy distribution that reaches <5% of the lung surface area and imposes limits on the maximum dose volume to avoid severe stress, hypoxia or death. Instillation cannot be used to deliver dry powder.3,5,8

By contrast, inhalation systems for rodents incorporate many of the same inhaler and nebulizer technologies used in clinical practice. These produce particles in a respirable range, resulting in more uniform lung distribution and greater parity between experimental and clinical results.2,3 Disadvantages of inhalation methods center on the cost, difficulty in determining the delivered dose, very low delivery efficiencies, loss of sample material inside the apparatus or on the fur or nasal passages of the animal with the added risk of systemic drug absorption transdermally or orally by grooming.5,9 Technologies that depend on propellants or compressed gas to create particles in a respirable range also impart high momentum and shear stresses that can increase impaction prior to reaching the lungs and damage viability, particularly with bioactive materials.5

**Intratracheal aerosolization: A hybrid approach**

Both the pros and cons associated with instillation and inhalation led to development of a hybrid approach designed to capture the best features of each. Intratracheal aerosolization is a form of drug delivery in which an atomizer located in the distal tip of a long, narrow, stainless steel or plastic tube is used to administer an aerosol directly to the lungs when inserted down the trachea, above the carina or beyond, in an anesthetized animal. For preclinical studies, there are currently two commercially available technologies for liquid intratracheal aerosol administration and one for dry powders.

One such liquid device is a “nebulizing catheter” from Trudell Medical International (London, Ontario, Canada), named the AeroProbe, in which liquid is conveyed down a tube through a central lumen and pressurized gas is conveyed to peripheral lumens from a compressed air source of about 50 psi. The close proximity of the liquid and gas lumens at the distal tip produces an aerosol in a range of 20 – 40 µm.10

By contrast, an air-free liquid device and an air-driven dry powder device are available from Penn-Century, Inc. (Wyndmoor, PA, US), which are designed specifically for preclinical use. These devices have been cited in more than 1,100 published in vivo, ex vivo and in vitro studies that
employ intratracheal aerosolization. The following is a detailed discussion of these devices.

**Preclinical tools designed for intratracheal aerosolization of liquids and dry powders**

The liquid and dry powder devices were designed, developed and patented in the 1990s by company founder, Theodore J. Century, PhD, of Philadelphia, PA. They have been adopted worldwide by pharmaceutical, biotech, academic and government research settings in 39 countries. The devices are manually operated, purely mechanical, sterilizable and reusable. They were designed to combine the type of control, speed, precise dose quantification and efficient use of sample material achieved by instillation methods with a particle size range small enough to achieve the broad uniform distributions possible with nebulization. The devices permit the user to rapidly dispense a precise aerosol dose, in liquid or dry powder form, directly to the lungs with delivered dose efficiencies as high as 90-100%.11,12

Soon after these liquid devices were introduced, the company developed the only intratracheal technology for administration of dry powders. The Dry Powder Insufflator (Figure 2) permits targeted, precisely quantifiable and efficient powder delivery to the lungs, in contrast with exposure models. The device consists of a sample chamber that holds a small, precisely-weighed dose of powder away from ambient air. By applying small puffs of air from an air syringe or air pump, valves inside the device insufflate the powder and send it down a hollow delivery tube directly into the lungs. The device has been used for delivery of nano- to macro-scale particles, including bioactive compounds. Powders have been shown to be unaltered by passage through the device and able to achieve uniform distribution and high concentration.5,17

Initially, Penn-Century introduced the MicroSprayer Aerosolizer (Figure 1), a totally air-free atomizer designed specifically for intratracheal delivery of liquid formulations. It consists of a long, thin, stainless steel tube with a sub-miniaturized, aerosol generator located in the tip which produces a low momentum, highly concentrated cloud of aerosol when operated with a gas-tight syringe. It has been successful in aerosolizing a vast array of small and macromolecule solutions and suspensions, including cells. Manufactured in two models, one more narrow and flexible than the other, each produces a particle size range (19–22 µm in the smaller model, 25–30 µm in the larger model) that has been demonstrated to reach the lung periphery down to the alveoli in species from mice to primates, and to achieve high concentration and uniform deposition when the tip of device is correctly placed in situ.11,13-16

These intratracheal liquid and dry powder technologies can be made to any length, and have been used in a range of experimental animals, including mice, rats, guinea pigs, rabbits, chickens, ferrets, dogs, primates, sheep, cows and horses, as well as in *ex vivo* perfused lung models, intranasal studies and various *in vitro* laboratory set-ups. The intratracheal portion of the tip is intended to be inserted directly down the trachea to the carina in small animals (Figure 3), or introduced via an endotracheal tube or bronchoscope in larger animals. A number of researchers have also used the devices to apply aerosols to cell cultures, cascade impactors, particle sizing equipment, ventilation apparatus or improvised lab set-ups.
Applying intratracheal aerosol models to disease, drug delivery, imaging and treatment

Initially, researchers in the early 1990s used the MicroSprayer Aerosolizer to administer aerosolized solutions of lipopolysaccharide directly to the lungs of rats as a way of producing a more uniform model of adult respiratory distress syndrome than was possible by liquid bolus instillation and without the air burden associated with nebulizers.\textsuperscript{18,19}

Scientists soon began to exploit both liquid and dry powder devices to produce robust models of allergy, inflammation, lung injury and pulmonary fibrosis, as well as to mimic viral, fungal and bacterial diseases or to study the effects of pulmonary exposure to environmental and occupational toxins and biowarfare agents.

Scientists also applied intratracheal aerosol technologies to the development of vaccines and therapies for a wide range of pulmonary and systemic diseases, as well as diagnostic and imaging agents intended for oral inhalation. Local aerosol delivery of dyes and radio-opaque or fluorescent contrast materials have enabled researchers to validate lung deposition and dose concentration by region (Figure 4) or measure time-of-flight or other aerodynamic properties of test compounds.\textsuperscript{20,21}

Preclinical investigators have played an essential role in refining and standardizing the techniques and protocols for safe, effective use of intratracheal aerosol delivery, making it easier for others to follow. While these intratracheal devices are relatively simple in appearance, they require skill for safe and accurate placement in the trachea and optimal deposition in the lung, as well as familiarity with intubation and anesthesia protocols in laboratory animals. Once mastered, the devices have been shown to permit faster administration of a wider range of dose volumes and materials at higher concentrations than can be achieved with inhalation methods. For example, a 1 ml liquid dose that takes 5 minutes to aerosolize by air-jet nebulizer can be administered in less than 3 seconds by a MicroSprayer Aerosolizer – Model IA-1B, which is air-free.\textsuperscript{22}

When a liquid suspension of polystyrene microparticles was administered to cell cultures, concentration on the cell surface was observed to be 700 times greater when delivered with the air-free MicroSprayer Aerosolizer – Model IA-1C than by an air-jet nebulizer.\textsuperscript{25}
Pushing the capabilities of intratracheal drug delivery

From the beginning, scientists have pushed the capabilities of Penn-Century’s intratracheal aerosol technologies far beyond their original uses, using them to challenge prevailing assumptions about which drugs make suitable candidates for pulmonary delivery. By giving researchers greater control over dose volume, speed and lung deposition, these new tools helped foster innovation at every stage of drug discovery and development, including screening, pharmacokinetic and pharmacodynamic modeling and safety and efficacy assessment.

In the many publications and drug patents that cite the use of the devices, researchers have posed a host of intriguing questions that were not possible to ask with instillation and inhalation approaches. A few of these questions are highlighted below.

What is the optimal dose volume? Expanding and defining the dose response curve

Often, the most therapeutically effective dose of a given formula is far smaller or larger than is safe or feasible to deliver by instillation or inhalation. Intratracheal aerosol delivery technologies by Penn-Century have been shown to significantly expand the range of possible dose volumes that can be safely and rapidly administered to the lungs in aerosol form. These data can give researchers access to a clearer and broader picture of the dose response curve:

- **When more is better.** To achieve a more robust mouse model of pulmonary fibrosis, researchers have administered 200 µl of aerosolized bleomycin to mouse lungs using a MicroSprayer Aerosolizer, a dose volume that is four times greater than is considered safe by liquid bolus instillation.²⁴

- **When less is better.** In a rat model of stroke injury, pulmonary pretreatment with a 2 mg dose of flurbiprofen, a COX-1 and COX-2 inhibitor, was delivered intratracheally by a Dry Powder Insufflator and conferred greater protection and higher plasma levels than a 50 mg oral dose.²⁵

- **When systemic toxicity is a concern.** In a study of aerosolized chemotherapy administration in a mouse model of lung cancer, researchers using the MicroSprayer Aerosolizer observed that when they reduced the larger initial dose volume, they avoided systemic toxicity, significantly inhibited tumor growth and achieved a 50-fold greater chemotherapy concentration in the lung at less than half the dose volume required for standard intravenous delivery.²⁶

Can biologic materials be aerosolized and maintain integrity and viability?

Aerosol mechanisms requiring compressed air, heat or vibrating mesh to generate particles within a respirable size range may create shear stresses that disrupt or compromise the viability of biologically-based vaccines, therapies or models of disease. The MicroSprayer Aerosolizer and Dry Powder Insufflator have permitted researchers to successfully aerosolize a broad range of materials including liposomes, oligonucleotides, peptides, proteins, siRNA, monoclonal antibodies and live cells, as well as viral and bacterial disease agents without compromising their viability:

- In a primate model of cystic fibrosis, researchers were able to efficiently deliver aerosolized, radio-labeled, adeno-associated viral (AAV) vector to the lungs of Rhesus macaques and measure gene transfer and expression. Inserting a custom length MicroSprayer Aerosolizer via a bronchoscope, they achieved lung deposition of 93%, compared to less than 2% by laryngeal mask, and observed gene expression of 62–83%, compared with only 0.5% by nebulized mouthpiece.¹¹,¹³

- In a mouse model of respiratory challenges with influenza viruses, authors used a MicroSprayer Aerosolizer to administer a vaccine formulation of plasmid DNA formulated with the polymer polyethyleneimine (PEI-DNA) and observed an immediate and robust immune response in antigen-specific CD8(+) T cells.²⁷

- Researchers sprayed live cells during in vitro studies. In one study, authors reported that ultrasonic, jet or vibrating mesh methods were “inapplicable for spraying of living cells,” while the MicroSprayer Aerosolizer Model IA-1B preserved cell integrity and viability.²⁸ In another study, HeLa cells did not survive aerosolization by MicroSprayer Aerosolizer, but skin-derived fibroblast cells proliferated rapidly after spraying, forming a confluent monolayer.²⁹

Is it possible to obtain both uniform lung deposition and high concentration?

Aerosol delivery of dyes, fluorescent particles or radio-opaque methods using these intratracheal devices, have provided detailed confirmation of high concentrations and uniform local and regional lung deposition down to the lung periphery, using liquids or dry powders:

- In the primate model of gene therapy noted above, intratracheal aerosol delivery resulted in a...
50- to 100-fold enhancement in aerosol deposition compared with oral nebulization methods. Uniform distribution between the right and left lungs was also observed.\(^\text{11-13}\)

- In a range of lung imaging studies, the Dry Powder Insufflator has been used to deliver fluorescent particles. Authors observed deposition in the upper and lower airways of the rat,\(^\text{30}\) as well as down to the alveoli.\(^\text{16}\) In multiple studies in mice, intratracheal insufflation has been reported to achieve reproducible, deep lung deposition of >90% of the powder sample, “an amount far greater than possible with intranasal methods.”\(^\text{20}\)

- In a mouse model of direct protein transfection, researchers administered a liposomal protein suspension of β-galactosidase using a MicroSprayer Aerosolizer and demonstrated uniform deposition of the injected protein throughout the airways and the alveoli of mice down to the cellular level.\(^\text{31}\)

**How closely can in vivo disease models mirror physiological presentation in humans?**

Penn-Century devices have been used to induce robust, uniform in vivo models of infection and chronic diseases, as well as exposure models of toxicological threats:

- Military researchers used a MicroSprayer Aerosolizer via a bronchoscope to produce a “classic” dose-dependent, orthopox disease, consistent with inhalation of monkeypox virus. Intratracheal delivery required a fraction of the dose compared with intravenous delivery and greatly reduced the risk of aerosol exposures to lab members.\(^\text{32}\)

- Researchers administering bleomycin with a MicroSprayer Aerosolizer in a mouse model of pulmonary fibrosis observed uniformity in the entire lung field, with no difference between right and left lung or upper and lower sectors of the lung. They produced long lasting fibrotic lesions, principally in the subpleural regions that cause clinical problems in humans.\(^\text{35}\)

**What can antibiotics achieve when delivered to the lung in high concentrations?**

Inhaled antibiotic administration offers a rapid, non-invasive route for addressing infectious diseases but demonstrating efficacy can require greater lung concentrations and more timely delivery than is safe or feasible by in vivo inhalation or liquid bolus instillation. Intratracheal methods have been used for both vaccines and therapies against a range of bacterial and viral infection models, often permitting researchers to achieve greater efficacy more quickly at smaller doses:

- In a mouse model of fatal pneumococcal pneumonia, a MicroSprayer Aerosolizer was used to administer a bacteriophage, endolysin Cpl-1. Authors reported this efficiently reduced pulmonary bacterial counts and averted bacteraemia, leading to a reduction in mortality of 80%, despite a transient increase in inflammatory cytokines.\(^\text{34}\)

- In a mouse model of Pseudomonas aeruginosa, researchers found that aerosol administration by MicroSprayer Aerosolizer of a proprietary formulation of levofloxacin resulted in AUCs and Cmaxs that were 9-fold and 30-fold higher than those achieved with dose-normalized intraperitoneal injection.\(^\text{35}\)

**Conclusion**

Investigators have redefined the possibilities of preclinical pulmonary research through innovative use of intratracheal aerosol technologies introduced by Penn-Century. By offering greater control at the preclinical stage over the speed, dose volume and quantification of the delivered dose, preclinical researchers have been able to greatly expand the clinical relevance of preclinical pulmonary research.

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