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Direct Tracheal Instillation of Solutes into Mouse Lung

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Abstract

Intratracheal instillations deliver solutes directly into the lungs. This procedure targets the delivery of the instillate into the distal regions of the lung, and is therefore often incorporated in studies aimed at studying alveoli. We provide a detailed survival protocol for performing intratracheal instillations in mice. Using this approach, one can target delivery of test solutes or solids (such as lung therapeutics, surfactants, viruses, and small oligonucleotides) into the distal lung. Tracheal instillations may be the preferred methodology, over inhalation protocols that may primarily target the upper respiratory tract and possibly expose the investigator to potentially hazardous substances. Additionally, in using the tracheal instillation protocol, animals can fully recover from the non-invasive procedure. This allows for making subsequent physiological measurements on test animals, or reinstallation using the same animal. The amount of instillate introduced into the lung must be carefully determined and osmotically balanced to ensure animal recovery. Typically, 30-75 μL instillate volume can be introduced into mouse lung.

Protocol

1. Anesthesia

1. Anesthetizing mice with isoflurane may facilitate animal handling and restraint prior to administering anesthesia intraperitoneally. Using an inhaled anesthetic may or may not be suitable for a particular study: determine first the impact of using isoflurane, or any other anesthetic, on proposed lung study.
2. Anesthetize animals with isoflurane. The video shows the use of an isoflurane vaporizer chamber set at 2% isoflurane mixed with oxygen. If you do not have an isoflurane chamber, you can alternatively use standard open-drop exposure techniques. Enclose rats in a bell jar (or other appropriate non-porous container) with a wire mesh fitted to the bottom. To a gauze pad placed beneath the mesh wire, apply a mixture of 30% v/v isoflurane in propylene glycol. The use of the mesh ensures that the animal does not come into contact with the isoflurane-soaked pad, which could cause skin irritation and potential overdosing. After short exposure to isoflurane, the animal should be anesthetized and will not respond with a righting reflex when the jar is tipped and will not respond after toe pinch.
3. Further sedate mice with an intra-peritoneal injection of a mix of Xylazine and Ketamine. (It is good practice when performing intraperitoneal injections to draw back to insure that portions of the gut or other vital organs have not been penetrated). Mix 150 μL Xylazine (100 mg/mL) with 1000 μL Ketamine (100 mg/mL) in 8850 μL of 0.9% sterile phosphate buffered saline (PBS). For mice, the dosage will be approximately 80 μL per 10 gm of body weight. The final concentrations will be 10 mg/Kg Xylazine and 100 mg/Kg Ketamine. The animals should be kept dry and insulated to prevent excessive loss of body heat. Ophthalmic ointment can be applied to the eyes to prevent drying of the corneas.

2. Preparation of Surgical Area

1. Shaving the area where the surgical incision will be made prevents contamination from non-prepped areas. An electric razor can closely shave the fur off the animal. The shaved area should be larger than the incision site.
2. Subsequent application of hair removal cream, such as Nair, will remove all remaining fur. Apply a thick layer of hair removal cream to the surgical area and leave on for 3 minutes. Wipe away the fur and hair removal cream with a damp towel. Do not leave the hair removal cream on in excess of 10 minutes.
3. Use 70% alcohol, or an alternative scrub solution, to aseptically prepare the surgical area.

3. Surgical Procedure

1. Position the anesthetized animal onto a sloped surgical board, or angled restraining stand.
2. Make a small incision near the anterior aspect of the neck (throat region).
3. Dissect away the platysma and anterior tracheal muscles in order to visualize and access the tracheal rings.
4. Typically, intratracheal injection volumes are 3 μl per gram of weight (approximately 30-75 μl final volume). Administer the appropriate amount of instillate using a 1mL subcutaneous (sub-Q) syringe with a 30 gauge, 5/16-inch needle. Hold the syringe bevel side up and parallel with the trachea, and inject the full volume of instillate into the trachea.
5. The animal may respond by gasping following the instillation of solute into the lungs. Introducing air through the syringe and observing whether the lungs expand will also confirm successful intubation techniques.
6. Remove syringe from the trachea.

4. Sutures.

1. Grasp the needle's swage (thickest portion of the needle where the suture material is attached) with a needle holder.
5. Animal Recovery

1. Animals should fully recover from the anesthesia and surgical procedure after 3-24 hours.

6. Representative Results

Intratracheal instillation of fluorescent compound, Cy5.5 conjugated to dextran, shows successful instillation of fluorescent compound in B6 mouse lung (Figure 1). The fluorescent dye is primarily targeted to the lung and is evenly distributed. Fluorescent signal in the gastrointestinal tract immediately following the procedure indicates that the esophagus was inadvertently intubated during the procedure (Figure 2). Investigators may also verify targeted delivery of compounds into the lung, and check distribution of the instillate, following the procedure by inflating the lungs and preparing lung tissue slices (2; 3) or fixed histological samples.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Fluorescent Cy5.5 signal co-registered with X-ray image in successfully instilled mouse lung. 1.4 mg Cy5.5 Dextran (at a concentration of 10 μg/μL) was instilled into the trachea of B6 mouse lung. Cy5.5 signal was detected using a 675 nm excitation filter and a 695 nm emission filter.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Co-registration of fluorescent signal shown with X-ray overlay in poorly instilled mouse. Inappropriate intubation of the esophagus during tracheal instillation protocol results in Cy5.5 delivery to the intestines.

**Discussion**

Intratracheal instillations have been used in several various studies to evaluate the toxicity of test compounds (6), induce alveolar lung injury (4; 7), replace surfactants (8), as well as alter gene expression via delivery of small oligonucleotides directly into the lung (5). We are currently exploring the use of instilling 1) fluorescent redox-sensitive material into the lung in order to measure oxidative stress in vivo under normal and pathophysiological conditions, as well as 2) instilling fluorescent indicators with pharmacological compounds, DNA vectors, viruses, and/or microRNA to assess the effect of each instillate on lung fluid transport.

There are several advantages of introducing foreign material into the lung using an instillation protocol over inhalation exposure (reviewed in (1)). The primary benefits of using an instillation protocol includes limiting exposure to toxic, carcinogenic, or radioactive compounds. Additionally, solids as well as liquid material, can be introduced into the lung. Arguably, the greatest drawback to using the tracheal instillation approach is that the introduction of the instillate is invasive and nonphysiological.

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