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Title: Halogenated Agent Delivery in Porcine Model of Acute Respiratory Distress Syndrome via an Intensive Care Unit Type Device

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   X Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **50**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Raiko Blondonnet**: This model can help to improve our understanding of the mechanisms involved in lung injury and to test halogenated agents as potential novel therapies for acute respiratory distress syndrome **[1]**.

REQUIRED:

1.2. **Matthieu Jabaduon**: The main advantage of this technique is that halogenated agents are delivered by an anesthetic conserving device, such as those used for ICU patients **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Mattieu Jabaudon**: Our technique uses a clinically relevant device to deliver inhaled ICU sedation, facilitating novel translational approaches for the study of the effects of halogenated agents in ARDS **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Animal Ethics Committee of the French Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche.

Protocol

2. Animal Preparation

2.1. After confirming a lack of response to pedal reflex in a 2-4-month old, 10-15-kilogram, white, male, Landrace piglet **[1-TXT]**, use surgical exposure of the right internal jugular vein and the Seldinger method to insert a 3-lumen catheter into the vessel for central venous access **[2]**.

2.1.1. WIDE: Talent checking pedal reflex Videographer: More Talent than pig in shot
TEXT: Anesthesia: propofol 3 mg/kg + sufentanil 0.3 micrograms/kg i.v.

2.1.2. Catheter being inserted

2.2. Make a cutaneous midline incision on the ventral aspect of the neck 2 centimeters lateral from the trachea **[1]** and use surgical forceps to dissect the tissues **[2]**.

2.2.1. Incision being made *Videographer: Difficult step*
2.2.2. Tissue being dissected *Videographer: Difficult step*

2.3. After using an 18-gauge needle to make a puncture in the internal jugular vein in a craniocaudal direction, insert a 60-centimeter piece of 0.81-millimeter-diameter "J" guidewire through the needle **[1]** and quickly but carefully replace the needle with a venous catheter with three lines along the guidewire **[2]**.

2.3.1. Shot of needle, then guidewire being inserted through needle *Videographer: Difficult step*
2.3.2. Needle being removed/catheter being placed *Videographer: Difficult step*

2.4. Then remove the guidewire while keeping the catheter in place **[1]**.

2.4.1. Guidewire being removed

2.5. With the right forelimb of the piglet in extension, make a cutaneous incision on the right groin area of the piglet **[1]** and use surgical forceps to dissect the subcutaneous and muscular tissues **[2]**.

2.5.1. Incision being made
Tissue being dissected

2.6. Then use surgical exposure of the right femoral artery and the Seldinger method to insert a 20-centimeter 3-5-French thermodilution catheter as an arterial line **[1]**.

2.6.1. Catheter being inserted

3. Acid-Induced Acute Lung Injury

3.1. For acid-induced acute lung injury, using the anatomical landmark of the last segment of the sternum **[1]**, measure the distance between the tip of the endotracheal tube and the carina of the piglet **[2]**.

3.1.1. WIDE: Talent identifying landmark *Videographer: More Talent than pig in shot*
3.1.2. Distance being measured

3.2. Use a black pen to mark this distance on a size 14 suction catheter **[1]** and insert the catheter through the endotracheal tube up to the landmark **[2]**.

3.2.1. Distance being marked
3.2.2. Catheter being inserted

3.3. Then gently instill 4 milligrams/kilogram of 0.05-molar hydrogen chloric acid through the suction catheter for a period of 3 minutes **[1]** before removing the catheter **[2]**.

3.3.1. Acid being instilled *Videographer: Difficult step*
3.3.2. Catheter being removed *Videographer: Difficult step*

4. Mechanical Ventilation and Halogenated Anesthetics

4.1. After removing the catheter, set an intensive care ventilator to deliver volume-controlled ventilation **[1]** at a tidal volume of 6 milligrams/kilogram, a positive end-expiratory pressure of 5 centimeters of water, and an inspired oxygen fraction of 40% **[2]**.

4.1.1. WIDE: Talent setting up ventilator
4.1.2. Shot of ventilator settings being set up as narrated/set as narrated *Videographer: Difficult step*

4.2. Next, attach the appropriate filling adapter to a 250-milliliter bottle of the halogenated agent of interest **[1]** and attach a 60-milliliter syringe to the adapter **[2]**.

4.2.1. Talent attaching adapter to bottle
4.2.2. Talent attaching syringe to adapter

4.3. Turn the bottle upside down **[1]** and retract the plunger to fill the syringe with the agent **[2]**.

4.3.1. Talent turning bottle upside down
4.3.2. Plunger being retracted

4.4. Turn the bottle upright and remove the syringe **[1]** and place a charcoal filter close to the ventilator **[2]**.

4.4.1. Talent removing syringe from upright bottle
4.4.2. Talent placing filter to ventilator

4.5. Remove the protective cap from the charcoal filter **[1]** and use a flex tube to connect the filter to the expiratory valve of the ventilator **[2]**.

4.5.1. Talent removing cap
4.5.2. Talent connecting filter to valve

4.6. Connect an ionomer membrane dryer line to the gas sampling port of an anesthetic conserving device **[1]** and connect one side of the gas sampling line to the ionomer membrane dryer line **[2]**.

4.6.1. Talent connecting line to port
4.6.2. Talent connecting sampling line to dryer line

4.7. Connect the other side of the gas sampling line to the gas analyzer **[1]** and insert the anesthetic conserving device between the Y-piece of the respiratory circuit and the endotracheal tube **[2]**.

4.7.1. Line being connected to analyze
4.7.2. Device being inserted

4.8. Ensure that the anesthetic conserving device is black side-up and sloped toward the piglet **[1]** and deliver inhaled sedation through the anesthetic conserving device **[2]**.

4.8.1. Talent adjusting device *Videographer: More Talent than piglet in shot*
4.8.2. Talent starting sedation *Videographer: More Talent than piglet in shot*

4.9. Place the syringe in the syringe pump **[1]** and connect the anesthetic agent line to the syringe **[2]**.

4.9.1. Talent placing syringe into pump *Videographer: Difficult step*
4.9.2. Talent connecting line to syringe *Videographer: Difficult step*

4.10. Prime the agent line with a 1.5-milliliter bolus of the halogenated agent **[1]** and set the pump to the appropriate initial pump rate to 3 milliliters per hour of isoflurane or 5 milliliters per hour of sevoflurane **[2-TXT]**.

4.10.1. Line being primed with bolus
4.10.2. Talent adjusting pump rate

4.11. Confirm that the gas analyzer displays an expired halogenated agent fraction or equivalent minimal alveolar concentration value greater than zero **[1]**, giving an additional 300-microliter bolus of halogenated agent as necessary **[2]**.

4.11.1. Shot of analyzer showing appropriate concentration value *Videographer: Difficult step*
4.11.2. Talent giving additional bolus *Videographer: Difficult step*

4.12. Then adjust the syringe pump rate as necessary to reach the appropriate concentration depending on the minute volume and targeted concentration **[1-TXT]**, continuing to administer the appropriate anesthetic fractions throughout the experiment **[2]**.

4.12.1. Talent adjusting pump rate **TEXT: *i.e.*, 2-7 mL/h = 0.2-0.5% isoflurane; 4-10 mL/h = 0.5-1.4% sevoflurane**
4.12.2. Talent delivering fraction(s)

5. Monitoring

5.1. Use the external monitor to collect heart rate, blood pressure, and peripheral oxygen saturation measurements **[1]** and record the tidal volume, respiratory rate, set and auto-positive end-expiratory pressure, compliance of the respiratory system, airway resistance, inspiratory plateau pressure, peak inspiratory pressure, and driving pressure as measured by the ventilator **[2]**.

5.1.1. WIDE: Talent checking monitor
5.1.2. Shot of measurement readouts

5.2. Use the Nitrogen Wash In-Wash Out method to calculate the lung functional residual capacity **[1]** and use the thermal indicator to measure the extravascular water volume of the lungs, cardiac index, and systemic vascular resistance **[2]**.

5.2.1. Talent at computer or bench, calculating lung functional residual capacity
5.2.2. Shot of thermal indicator output

5.3. To measure the net alveolar fluid clearance rate, insert a soft 14-French suction catheter through the endotracheal tube into a wedged position in the distal bronchus **[1]** and apply gentle suction to collect an undiluted pulmonary edema fluid sample **[2]**.

5.3.1. Catheter being inserted *Videographer: Difficult step*
5.3.2. Suction being applied *Videographer: Difficult step*

5.4. For mini bronchoalveolar lavage sampling, instill 50 milliliters of a 0.9% sodium chloride solution into the suction catheter **[1]** and collect the resulting volume of lavage **[2]**.

5.4.1. NaCl being instilled into catheter *Videographer: Difficult step*
5.4.2. Sample being collected *Videographer: Difficult step*

5.5. For blood gas analysis, collect arterial blood gases through the arterial line in a 3-milliliter preset syringe with a Luer-Lok tip at baseline **[1]** and use a point-of-care blood gas analyzer to immediately measure the acute respiratory distress syndrome-partial pressure of arterial oxygen ratio, partial pressure of carbon dioxide, pH, serum lactate, and serum creatinine levels **[2-TXT]**.

5.5.1. Talent collecting gas
5.5.2. Talent using analyzer to obtain measurements **TEXT: Repeat measurements 1/h/4h after acid instillation**

5.6. At the end of the experiment, harvest the whole lung tissue into alcohol acetified formalin for macro- and histologic tissue analysis **[1]**.

5.6.1. Talent placing lung into fixative OR Shot of lung in fixative OR LAB MEDIA: Figure 7

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.3., 4.1., 4.9., 4.11., 5.3., 5.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

2.2., 2.3. Indeed, the placement of both venous and artery catheters is at risk of wounding the vessels during the puncture, of development of hemorrhagic shock if blood loss is too important, and the animals could ultimately die. Hopefully, our team has a strong experience in large animal models of ARDS and training and preparation are always required to perform these procedures, such as in the piglet model described in this video.

Results

6. Results: Representative Porcine Acute Respiratory Distress Syndrome (ARDS) Evaluation

6.1. In this representative experiment, a two-way repeated-measures analysis of variance **[1]** indicated a significant time by group interaction with a detrimental effect of hydrogen chloride-induced ARDS (A-R-D-S) on the acute respiratory distress syndrome-partial pressure of arterial oxygen ratio **[2]** compared to sham animals without ARDS **[3]**.

6.1.1. LAB MEDIA: Figure 3
6.1.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize HCl data line and data bars*
6.1.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize SHAM data line and data bars*

6.2. A significant between-group difference was noted in the undiluted pulmonary edema fluid levels of the total protein 4 hours after mechanical ventilation **[1]**, with an association observed between hydrogen chloride-induced ARDS and increased bronchoalveolar protein levels **[2]** compared to that observed in sham animals **[3]**.

6.2.1. LAB MEDIA: Figure 4 *Video Editor: please add/emphasize $P<10^{-4}$ text*
6.2.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize HCl data bar*
6.2.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize SHAM data bar*

6.3. Two-way repeated-measures analysis of variance also revealed a significant time by group interaction **[1]** between hydrogen chloride-induced ARDS and increased extravascular lung water **[2]** compared to sham animals **[3]**.

6.3.1. LAB MEDIA: Figure 5A
6.3.2. LAB MEDIA: Figure 5A *Video Editor: please emphasize HCl data line*
6.3.3. LAB MEDIA: Figure 5A *Video Editor: please emphasize SHAM data line*

6.4. Cardiac output demonstrated an increased trend in acid-injured animals **[1]**, while systemic vascular resistance values were slightly lower in the hydrogen chloride-induced ARDS group **[2]**.

6.4.1. LAB MEDIA: Figures 5B and 5C *Video Editor: please emphasize HCl data line in Figure 5B*
6.4.2. LAB MEDIA: Figures 5B and 5C *Video Editor: please emphasize SHAM data line in Figure 5C*

6.5. Four hours after injury, macroscopic lung damage **[1]**, including visible hemorrhage and congestion, can be observed within the red regions of lungs harvested from hydrogen chloride-induced ARDS animals **[2]** that is absent in untreated piglet lung tissue **[3]**.

6.5.1. LAB MEDIA: Figures 7A and 7B

6.5.2. LAB MEDIA: Figures 7A and 7B *Video Editor: please emphasize white arrows/regions indicated by white arrows*

6.5.3. LAB MEDIA: Figures 7A and 7B *Video Editor: please emphasize Figure 7A image*

6.6. Histologic analysis reveals a greater cellularity within acid-injured lung tissue slices **[1]** with more areas of atelectasis and increased alveolar disruption, hyaline membranes, protein debris, hemorrhage **[2]**, and alveolar wall thickening **[4]**.

6.6.1. LAB MEDIA: Figures 7C and 7D

6.6.2. LAB MEDIA: Figures 7C and 7D *Video Editor: please emphasize black arrowhead/cells indicated by black arrowhead in Figure 7D*

6.6.3. LAB MEDIA: Figures 7C and 7D *Video Editor: please emphasize white arrow/tissue indicated by white arrow in Figure 7D*

6.6.4. LAB MEDIA: Figures 7C and 7D *Video Editor: please emphasize black arrow/tissue indicated by black arrow in Figure 7D*

6.7. These disruptions are absent in sham samples **[1]**.

6.7.1. LAB MEDIA: Figures 7C and 7D *Video Editor: please emphasize Figure 7C*

Conclusion

7. Conclusion Interview Statements

7.1. **Raiko Blondonnet**: In addition to administering halogenated volatiles, this model of acid induced ARDS could be useful for studying specific pathways of interest, such as those involved in lung epithelial injury and repair **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera