Journal of Visualized Experiments

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

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61644R2
Full Title:	Halogenated Agent Delivery in Porcine Model of Acute Respiratory Distress Syndrome via an Intensive Care Unit Type Device
Corresponding Author:	Matthieu Jabaudon, M.D., Ph.D. CHU Clermont-Ferrand, Université Clermont Auvergne, CNRS, INSERM Clermont-Ferrand, FRANCE
Corresponding Author's Institution:	CHU Clermont-Ferrand, Université Clermont Auvergne, CNRS, INSERM
Corresponding Author E-Mail:	mjabaudon@chu-clermontferrand.fr
Order of Authors:	Raiko Blondonnet
	Bertille Paquette
	Jules Audard
	Ridvan Guler
	François-Xavier Roman
	Ruoyang Zhai
	Corinne Belville
	Loïc Blanchon
	Thomas Godet
	Emmanuel Futier
	Jean-Etienne Bazin
	Jean-Michel Constantin
	Vincent Sapin
	Matthieu Jabaudon, M.D., Ph.D.
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Clermont-Ferrand, Auvergne-Rhône-Alpes, FRANCE
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please specify the section of the submitted manuscript.	
Please provide any comments to the journal here.	

Cover letter

Name of Corresponding Author:

BLONDONNET Raïko, MD, MSc (rblondonnet@chu-clermontferrand.fr)

Manuscript title :

Effects of halogenated agents delivered through an intensive care unit-type device in a porcine model of acute respiratory distress syndrome

Please find enclosed a revised method article that has never been submitted or published elsewhere. This is an experimental study with a background in previously published animal studies created by our group. We hope this manuscript will be of interest to you and the readers of the journal.

Here, we report our method to use a reproducible experimental animal model specifically designed to reach precise and controlled concentrations of sevoflurane or isoflurane, using the AnaConDa-S device, during mechanical ventilation in piglets with hydrochloric acid-induced lung injury. We have developed this model in order to improve our understanding of the mechanisms involved in lung epithelial injury and to test novel therapies for acute respiratory distress syndrome (ARDS), a frequent cause of respiratory failure and death in critically ill patients.

The current experimental model of ARDS, that induces substantial lung injury in larger animals than mice, has significant advantages such as rapid onset (within one hour in general), good reproducibility and stability over time, and a low mortality rate. This model is very relevant to further investigate the effects of inhaled halogenated agents on the lung and better assess their potential benefit in the management of patients with ARDS.

The research results we submit today to JOVE are part of a translational approach to better understanding the clinical effects of inhaled sevoflurane in patients with ARDS and in exploring the specific effects of sevoflurane in the lung using experimental models. Sevoflurane is a halogenated anesthetic that is widely used in the operating room around the world, and devices are now available in intensive care units to deliver inhaled sedation to patients (with sevoflurane, but also with other halogenates such as isoflurane or desflurane).

On behalf of my co-authors, I am submitting the enclosed material for possible publication in JOVE. It has not been submitted for publication or published, in whole or in part, elsewhere.

I attest to the fact that all authors listed on the title page have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to JOVE.

I acknowledge that both I and the other authors have read the Instructions for Authors and agree with its contents. I acknowledge that if the enclosed manuscript is part of a larger whole or if the primary analysis has been previously published, this must be explicitly stated in the manuscript and the previous publication cited.

No conflict of interest, other source of financial support, corporate involvement, patent holdings, *etc*. is to be declared for all authors.

Signature Date

May 11, 2020

BLONDONNET Raiko

TITLE:

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

AUTHORS AND AFFILIATION:

Raiko Blondonnet[1,2], Bertille Paquette[1,2], Jules Audard[1,2], Ridvan Guler[1,2], François-Xavier Roman[1,2], Ruoyang Zhai[2], Corinne Belville[2], Loïc Blanchon[2], Thomas Godet[1], Emmanuel Futier[1,2], Jean-Etienne Bazin[1], Jean-Michel Constantin[3], Vincent Sapin[2,4], Matthieu Jabaudon[1,2,5]

[1]Department of Perioperative Medicine, CHU Clermont-Ferrand, Clermont-Ferrand, France
[2] Université Clermont Auvergne, GReD, CNRS, INSERM, Clermont-Ferrand, France
[3]Sorbonne University, GRC 29, AP-HP, DMU DREAM, Department of Anesthesiology and Critical Care, Pitié-Salpêtrière Hospital, Paris, France
[4]Department of Biochemistry and Molecular Genetics, CHU Clermont-Ferrand, Clermont-Ferrand, France
[5]Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, USA

CORRESPONDING AUTHOR:

Raiko Blondonnet (rblondonnet@chu-clermontferrand.fr)

EMAIL ADDRESSES OF CO-AUTHORS:

Bertille Paquette (bpaquette@chu-clermontferrand.fr)
Jules Audard (jaudard@chu-clermontferrand.fr)
Ridvan Guler (rguler@chu-clermontferrand.fr)
François-Xavier Roman (fxroman@chu-clermontferrand.fr)
Ruoyang Zhai (ruoyang.zhai@uca.fr)
Corinne Belville (corinne.belville@uca.fr)
Loïc Blanchon (loic.blanchon@uca.fr)
Thomas Godet (tgodet@chu-clermontferrand.fr)
Emmanuel Futier (efutier@chu-clermontferrand.fr)
Jean-Etienne Bazin (jebazin@chu-clermontferrand.fr)
Jean-Michel Constantin (jean-michel.constantin@aphp.fr)
Vincent Sapin (vsapin@chu-clermontferrand.fr)
Matthieu Jabaudon (mjabaudon@chu-clermontferrand.fr)

KEYWORDS:

porcine model, sevoflurane, isoflurane, halogenated agents, lung injury, novel therapies, ARDS

SUMMARY:

We describe a model of hydrochloric acid-induced acute respiratory distress syndrome (ARDS) in piglets receiving sedation with halogenated agents, isoflurane and sevoflurane, through a device used for inhaled intensive care sedation. This model can be used to investigate the biological mechanisms of halogenated agents on lung injury and repair.

ABSTRACT:
Acute respiratory distress syndrome (ARDS) is a common cause of hypoxemic respiratory failure and death in critically ill patients, and there is an urgent need to find effective therapies. Preclinical studies have shown that inhaled halogenated agents may have beneficial effects in animal models of ARDS. The development of new devices to administer halogenated agents using modern intensive care unit (ICU) ventilators has significantly simplified the dispensing of halogenated agents to ICU patients. Because previous experimental and clinical research suggested potential benefits of halogenated volatiles, such as sevoflurane or isoflurane, for lung alveolar epithelial injury and inflammation, two pathophysiologic landmarks of diffuse alveolar damage during ARDS, we designed an animal model to understand the mechanisms of the effects of halogenated agents on lung injury and repair. After general anesthesia, tracheal intubation, and the initiation of mechanical ventilation, ARDS was induced in piglets via the intratracheal instillation of hydrochloric acid. Then, the piglets were sedated with inhaled sevoflurane or isoflurane using an ICU-type device, and the animals were ventilated with lung-protective mechanical ventilation during a 4 h period. During the study period, blood and alveolar samples were collected to evaluate arterial oxygenation, the permeability of the alveolar-capillary membrane, alveolar fluid clearance, and lung inflammation. Mechanical ventilation parameters were also collected throughout the experiment. Although this model induced a marked decrease in arterial oxygenation with altered alveolar-capillary permeability, it is reproducible and is characterized by a rapid onset, good stability over time, and no fatal complications.

We have developed a piglet model of acid aspiration that reproduces most of the physiological, biological, and pathological features of clinical ARDS, and it will be helpful to further our understanding of the potential lung-protective effects of halogenated agents delivered through devices used for inhaled ICU sedation.

INTRODUCTION:
Acute respiratory distress syndrome (ARDS) is a common cause of hypoxemic respiratory failure and death in critically ill patients[1]. It is characterized by both diffuse alveolar epithelial and endothelial injuries, leading to increased permeability and pulmonary edema, altered alveolar fluid clearance (AFC), and worsened respiratory distress[2]. The resorption of alveolar edema and recovery from ARDS require epithelial fluid transport through the alveoli to remain intact, which suggests that a therapy improving AFC could be useful[3,4]. Although lung-protective ventilation and a restrictive strategy for intravenous fluid therapy have proven beneficial in improving outcomes[2,5], they are still associated with high mortality and morbidity[6]. Therefore, there is an urgent need to develop effective therapies for the syndrome and to better understand the precise mechanisms through which such therapies might work.

Halogenated anesthetics, such as isoflurane or sevoflurane, have been widely used for general anesthesia in the operating room. Sevoflurane is associated with decreased inflammation in the lungs of patients undergoing thoracic surgery and with a decrease in postoperative pulmonary complications, such as ARDS[7]. Similar results have been found in a meta-analysis of patients after cardiac surgery[8]. Halogenated volatiles also have a bronchodilatory effect[9,10] and perhaps some properties that protect several organs, such as the heart[8,11] and the kidneys[12–14]. Recently, there has been growing interest in the clinical use of inhaled

anesthetics as sedatives in the intensive care unit (ICU). Both animal and human studies support the protective effects of pretreatment with halogenated agents before prolonged ischemia of the liver[15], the brain[16], or the heart[11]. Halogenated agents also have potential pharmacokinetic and pharmacodynamic advantages over other intravenous agents for the sedation of critically ill patients, including a rapid onset of action and fast offset due to little accumulation in tissues. Inhaled halogenated agents decrease intubation times in comparison with intravenous sedation in patients undergoing cardiac surgery[17]. Several studies support the safety and efficacy of halogenated agents in the sedation of ICU patients[18–20]. In experimental models of ARDS, inhaled sevoflurane improves gas exchange[21,22], reduces alveolar edema[21,22], and attenuates both pulmonary and systemic inflammation[23]. Isoflurane also ameliorates lung repair after injury by maintaining the integrity of the alveolar-capillary barrier, possibly by modulating the expression of a key tight junction protein[24–26]. In addition, mouse macrophages that were cultured and treated with isoflurane had better phagocytic effects on neutrophils than macrophages that were not treated with isoflurane[27].

However, the precise biological pathways and mechanisms accounting for the lung-protective properties of volatile anesthetics remain largely unknown to date, requiring further investigation[18]. Additional studies are also warranted to investigate the precise effects of sevoflurane on lung injury and to verify whether experimental evidence can be translated to patients. The first randomized control trial from our team found that the administration of inhaled sevoflurane in patients with ARDS was associated with oxygenation improvement and decreased levels of both pro-inflammatory cytokines and lung epithelial injury markers, as assessed by plasma and alveolar soluble receptors for advanced glycation end products (sRAGE)[28]. As sRAGE is now considered as a marker of alveolar type 1 cell injury and a key mediator of alveolar inflammation, these results could suggest some beneficial effects of sevoflurane on the lung alveolar epithelial injury[21,29,30].

The use of halogenated agents for inhaled ICU sedation has long required operating room anesthesia ventilators and gas vaporizers to be deployed in the ICU. Since then, anesthetic reflectors suitable for the use with modern critical care ventilators have been developed for specific use in the ICU[31]. These devices feature modified heat and moisture exchanging filters inserted between the Y-piece of the respiratory circuit and the endotracheal tube. They allow the administration of halogenated agents, with isoflurane and sevoflurane being the most frequently used, and they consist of a porous polypropylene evaporator rod, into which a liquid agent, delivered by a specific syringe pump, is released. The halogenated agent is absorbed during expiration by a reflecting medium contained in the device and it is released during the next inspiration, allowing recirculation of approximately 90% of the expired halogenated agent[31,32]. Recently, a miniaturized version of the device was developed with an instrumental dead space of 50 mL, making it even more suitable for use during ultra-protective ventilation in ARDS patients, with tidal volumes that could be as low as 200 mL[31]. Such a miniaturized device has never been studied in an experimental piglet model of ARDS.

Because previous research supports the promising roles of halogenated volatiles in lung alveolar inflammation and injury during ARDS, we designed an experimental animal model to achieve a translational understanding of the mechanisms of the effects of halogenated agents on lung injury and repair[33–35]. In this study, we developed a model of hydrochloric acid (HCl)-induced ARDS in piglets in whom inhaled sedation can be delivered using the miniaturized

version of the anesthetic conserving device, an ICU-type device. This large animal model of ARDS could be used to further our understanding of the potential lung-protective effects of inhaled halogenated agents.

PROTOCOL:

The study protocol was approved by the Animal Ethics Committee of the French *Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche* (approval number 01505.03) before being registered at preclinicaltrials.eu (*Pre-clinical registry identifier* PCTE0000129). All procedures were performed in the *Centre International de Chirurgie Endoscopique, Université Clermont Auvergne*, Clermont-Ferrand, France, in accordance with the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines[36].

1. Animal preparation and anesthesia

1.1. Piglet mode

1.1.1. Ensure that the experimental protocol is consistent with guidelines for animal experiments, including the 3R principles (replacement, reduction, and refinement) and national/international regulations.

1.1.2. Obtain approvals from the ethics committee for care and use of experimental animals at the relevant institution before starting the protocol.

1.1.3. Use a male white Landrace piglet (2–4 months old; weighing 10–15 kg).

1.1.4. Place the piglet in the supine position after premedication using intramuscular azaperone (described in 1.2.2).

1.2. Anesthesia induction

1.2.1. Restrict animals from having food for overnight while allowing free access to water.

1.2.2. Administer anxiolytic premedication to the piglet using intramuscular azaperone (2 $mg.kg^{-1}$) behind the ear.

1.2.3. Apply a finger pressure on the soft tissues of the auricular base of the piglet to identify the medial and lateral auricular vein.

1.2.4. Insert a peripheral intravenous 22 G catheter in the medial or lateral auricular vein of the piglet. Follow with the catheter at a shallow angle of 45˚ through the skin and advance until blood appears through the catheter.

1.2.4. Induce general anesthesia with intravenous propofol (3 $mg.kg^{-1}$) and sufentanil (0.3 $\mu g.kg^{-1}$)[37]. Check the depth of the anesthesia by lack of response to pedal reflex.

1.3. Tracheal intubation[38,39]

1.3.1. Prepare the laryngoscope using a size 4 straight Miller laryngoscope blade.

1.3.2. Pass the laryngoscope into the pharyngeal cavity and depress the tongue with the laryngoscope blade, making the epiglottis visible.

1.3.4. Visualize the vocal cords of the piglet prior to orotracheal intubation.

1.3.5. Insert a 6 mm internal diameter cuffed endotracheal tube.

1.3.6. Inflate the endotracheal tube cuff to reach a cuff pressure around 20–30 cmH_2O.

1.3.7. Fix the endotracheal tube to the piglet's nose with micropore surgical tape.

1.3.8. Connect to the ventilator and initiate mechanical ventilation following the settings described in section 3.

1.4. Sedation maintenance

1.4.1. Maintain anesthesia with continuous intravenous infusion of propofol (5 $mg.kg^{-1}.h^{-1}$) before acid-induced lung injury. The infusion of propofol will be stopped when halogenated agents are started.

1.4.2. Add a continuous intravenous infusion of remifentanil (10–20 $\mu g.kg^{-1}.h^{-1}$ = 0.15–0.33 $\mu g.kg^{-1}.min^{-1}$) for pain management.

1.4.3. Add continuous intravenous infusion of cisatracurium (0.2 $mg.kg^{-1}.h^{-1}$) for a neuromuscular blockade.

1.4.4. Keep the body temperature of the piglet at approximately 38 °C using warm blankets.

1.4.5. Monitor electrocardiogram activity, the peripheral oxygen saturation (SpO_2), and arterial pressure continuously using an external monitor.

1.5. Surgery

1.5.1. Insert central venous access using a surgical exposure of the right internal jugular vein and the Seldinger method to insert a 3-lumen catheter (7 French, 16 cm).

1.5.1.1. Make a cutaneous midline incision on the ventral aspect of the neck, 2 cm lateral from the trachea. Use surgical forceps to dissect the tissues.

1.5.1.2. Localize the internal jugular vein (approximately 1–2 cm deep, lateral to the internal carotid artery) and, using the needle (18 G, 6.35 cm), make a puncture with a craniocaudal direction orientation.

1.5.1.3. With the hand, insert the "J" guidewire (0.81 mm diameter, 60 cm) through the needle. Gently remove the needle and quickly insert a venous catheter with three lines into

the internal jugular vein along the “J” guidewire. Remove the “J” guidewire while maintaining the venous catheter in place.

1.5.1.4. Aspirate blood through each line of the venous catheter to remove the air from the different lines and flush with 5 mL of saline solution (0.9% NaCl) to rinse the three lines.

1.5.1.5. Suture the skin with a 3.0 non-absorbable suture thread following the continuous Lembert pattern and fix the catheter to the skin with a single stitch and triple knots on each lateral perforation of the central venous catheter.

1.5.2. Insert an arterial line via surgical exposure of the right femoral artery and use the Seldinger method to insert the thermodilution catheter (3–5 French, 20 cm).

1.5.2.1. Place the right forelimb of the piglet in extension.

1.5.2.2. Make a cutaneous incision on the right groin area of the piglet. Use surgical forceps to dissect the subcutaneous and muscular tissues.

1.5.3.3. Localize the right femoral artery by palpating the femoral pulse (approximately 3–4 cm deep) and, using the needle (19 G, 54 mm), make a puncture with a caudocranial direction orientation.

1.5.2.4. Insert the “J” guidewire through the needle. Gently remove the needle and quickly insert an arterial catheter into the femoral artery up along the guidewire. Remove the guidewire while maintaining the catheter in place.

1.5.2.5. Remove the air from the arterial catheter and flush with saline solution to rinse the line.

1.5.2.6. Suture the skin with a 3.0 non-absorbable suture thread following the continuous Lembert pattern and fix the catheter to the skin with a single stitch and triple knots on each lateral perforation of the arterial catheter.

1.5.2.7. Plug the catheter on an arterial line tubing to allow retrieval of serial blood samples and continuous hemodynamic monitoring (arterial pressure, cardiac index, and extravascular lung water, as indexed to body weight) with a pulse contour cardiac output monitor device.

2. Acid-induced acute lung injury

CAUTION: Use gloves and glasses during this step to avoid any risk of contact of the acid with the skin or the eyes)

2.1. Make 100 mL of HCl at 0.05 M and pH 1.4.

2.2. Using the anatomical landmark of the last segment of the sternum, measure the distance between the tip of the endotracheal tube and the carina of the piglet.

2.3. Report this distance with a black pen on a Ch14 suction catheter.

2.4. Insert the suction catheter through the endotracheal tube up to the black landmark.

2.5. Gently instill 4 $mL.kg^{-1}$ (bodyweight) of acid through the suction catheter for over 3 min.

2.6. Remove the suction catheter.

3. Mechanical ventilation

3.1. Use volume-controlled ventilation on an intensive care ventilator.

3.2. Use a tidal volume of 6 $mL.kg^{-1}$, a positive end-expiratory pressure (PEEP) of 5 cmH_2O, and an inspired oxygen fraction (FiO_2) of 40%.

3.3. Adjust the respiratory rate to maintain the end-tidal carbon dioxide between 35 and 45 mmHg.

NOTE: Based on previous studies[37,40,41], lung injury is considered established when the arterial oxygen tension (PaO_2)-to-FiO_2 ratio decreases to 25% from the baseline, approximately 1 h after airway HCl instillation.

4. Halogenated anesthetics

NOTE: Start sedation using halogenated anesthetics (sevoflurane or isoflurane) once acid-induced lung injury is achieved. The intravenous sedation using propofol should then be interrupted.

4.1. Filling the syringe (**Figure 1A**)**:** Attach the filling adapter provided by the manufacturer to the 250 mL bottle of the halogenated agent and a 60 mL syringe to the filling adapter. Turn the bottle upside down and fill the syringe by pushing and pulling the plunger. Turn the bottle upright and remove the syringe.

4.2. Scavenging (**Figure 1B**)

4.2.1. Place the charcoal filter, used to remove halogenated hydrocarbon anesthetic gases, close to the ventilator.

4.2.2. Remove the protective cap from the charcoal filter.

4.2.3. Connect the charcoal filter to the expiratory valve of the ventilator with a flex tube.

4.3. Use the anesthetic conserving device (device used for inhaled ICU sedation) **(Figure 1C)** as described below.

4.3.1. Connect the ionomer membrane dryer line to the gas sampling port of the anesthetic conserving device.

4.3.2. Connect one side of the gas sampling line to the ionomer membrane dryer line.

4.3.3. Connect the other side of the gas sampling line to the gas analyzer.

4.3.4. Insert the anesthetic conserving device between the Y-piece of the respiratory circuit and the endotracheal tube.

4.3.5. Ensure that the anesthetic conserving device has the black side up and is sloped down toward the piglet.

4.4. Deliver inhaled sedation through the anesthetic conserving device **(Figure 2)**.

4.4.1. Place the specific syringe in the syringe pump.

4.4.2. Connect the anesthetic agent line to the syringe.

4.4.3. Prime the agent line with a bolus of 1.5 mL of the halogenated agent.

4.4.4. Adapt the initial pump rate in $mL.h^{-1}$ (initial syringe pump rate settings of isoflurane and sevoflurane are 3 and 5 mL/h, respectively) to the targeted expired sevoflurane fraction ($F_E$sevo) or the expired isoflurane fraction ($F_E$iso) value, as displayed on the gas analyzer.

4.4.5. Ensure that the gas analyzer displays a $F_E$sevo %–$F_E$iso % or equivalent minimal alveolar concentration value greater than zero. If necessary, give an additional bolus of 0.3 mL of the halogenated agent.

4.4.6. Adapt the syringe pump rate necessary to reach a certain concentration depending on the minute volume and the targeted concentration, with rates of 2–7 $mL.h^{-1}$ and 4–10 $mL.h^{-1}$ being, in general, associated with expired fractions of 0.2%–0.7% and 0.5%–1.4% for isoflurane[42] and sevoflurane[28,43], respectively.

4.4.7. During the experiment, continue administration of the halogenated agents with $F_E$sevo and $F_E$iso targets of 0.8–1.1 and 0.5–0.8, respectively.

5. Measurements

5.1. Monitoring

5.1.1. Collect different parameters as measured by the external monitor: heart rate, blood pressure, and peripheral oxygen saturation.

5.1.2. Record parameters as measured by the ventilator: tidal volume, respiratory rate, set PEEP, auto-PEEP (by applying an expiratory hold maneuver of 5 s on the ventilator), compliance of the respiratory system, airway resistance, inspiratory plateau pressure (by applying an inspiratory hold maneuver of 2 s on the ventilator), peak inspiratory pressure, and driving pressure.

5.1.3. Calculate the lung functional residual capacity using the Nitrogen Wash In/Wash Out method if integrated in the ventilator.

5.1.4. Use the thermal indicator previously inserted in the femoral artery to measure the extravascular water volume of the lungs, cardiac index, and systemic vascular resistance.

5.2. Undiluted pulmonary edema fluid sampling to measure the net AFC rate.

5.2.1. Insert a soft 14 Fr suction catheter into a wedged position in the distal bronchus through the endotracheal tube.

5.2.2. Sample edema fluid into a suction trap by applying gentle suction.

5.2.3. Centrifuge all samples at 240 *x g* at 4 °C for 10 min in a refrigerated centrifuge.

5.2.4. Collect the supernatants.

NOTE: Total protein concentration in undiluted pulmonary edema fluid is measured with a colorimetric method. Because the rate of clearance of edema fluid from the alveolar space is much faster than the rate of protein removal, the net AFC rate was calculated as Percent AFC = 100 × [1 - (initial edema protein/final edema total protein)] and thereafter was reported as %/h[37]. Undiluted pulmonary edema fluid samples are collected from the animals at baseline and 4 h later, as previously described[34,44–49].

5.3. Mini bronchoalveolar lavage sampling.

5.3.1. Insert a soft 14 Fr suction catheter into a wedged position in a distal bronchus through the endotracheal tube.

5.3.2. Instill 50 mL of a 0.9% sodium chloride solution into the suction catheter.

5.3.3. Promptly sample the fluid into a suction trap.

5.3.4. Collect the mini bronchoalveolar lavage.

NOTE: Total protein concentration in mini BAL is measured with a colorimetric method and, for example, the levels of proinflammatory cytokines, such as TNF-α, IL-6, IL-1β, and IL-18, are measured using a multiplex immunoassay method. Samples are collected 4 h after the acid-induced lung injury.

5.4. Blood gas analysis

5.4.1. Collect arterial blood gases through the arterial line in a 3 mL BD Preset syringe with BD Luer-Lok tip at baseline. Immediately measure PaO_2/FiO_2, $PaCO_2$, pH, serum lactate, and serum creatinine using a point-of-care blood gas analyzer.

5.4.2. Repeat this step every hour for 4 h after acid instillation.

5.5. Lung sampling

5.5.1. Sacrifice the piglet with an intravenous injection of pentobarbital (150 $mg.kg^{-1}$) at the end of the experiment (4 h after acid-induced lung injury).

5.5.2. Dissect and remove the whole lungs. Fix with alcohol acetified formalin.

5.5.3. Embed in paraffin and slice at a 10 µm thickness.

5.5.4. Stain with hematoxylin and eosin.

NOTE: Histological evidence of lung injury can be assessed using a standardized histology injury score[50].

REPRESENTATIVE RESULTS:

For this experiment, 25 piglets were anesthetized and divided in two groups: 12 piglets in the untreated group (SHAM group) and 13 piglets in the acid-injured group (HCl group). No piglet died before the end of the experiment. A two-way repeated-measures analysis of variance (RM-ANOVA) indicated a significant time by group interaction ($P < 10^{-4}$) with a detrimental effect of HCl-induced ARDS on PaO_2/FiO_2, compared to sham animals without ARDS (**Figure 3**). A significant between-group difference was noted in the undiluted pulmonary edema fluid levels of the total protein measured after 4 h of mechanical ventilation ($P < 10^{-4}$). HCl-induced ARDS was associated with increased BAL protein when compared to the sham animals (**Figure 4**). A two-way RM-ANOVA indicated a significant time by group interaction ($P < 10^{-4}$) with HCl-induced ARDS being associated with increased extravascular lung water, compared to sham animals without ARDS (**Figure 5A**). Cardiac output and systemic vascular resistance values are reported in **Figure 5B** and **Figure 5C**, respectively. Inspiratory and expiratory fractions of sevoflurane measured in all animals are reported in **Figure 6**, and macroscopic evidence of histologic lung injury is shown in **Figure 7**.

FIGURE LEGENDS:

Figure 1: Illustration of the set up needed to administer sedation with halogenated volatile agents using the anesthetic conserving device. (**A**) Filling the specific syringe with the bottle adapter. (**B**) Scavenging the halogenated agents using the scavenging charcoal filter. (**C**) Assembling both the syringe pump and the gas analyzer with the anesthetic conserving device to use along with the ventilator.

Figure 2: Schematic representation of the connection of the anesthetic conserving device to the respiratory circuit of the ventilator. This includes the integration of the module to measure lung functional residual capacity.

Figure 3: Measurement of the alteration in arterial oxygenation. (**A**) Evolution of arterial oxygen tension (PaO_2) to inspired oxygen fraction (FiO_2) ratio in untreated piglets (SHAM group, N = 12) and acid-injured piglets (HCl group, N = 13) during a 4 h period. (**B**) Evolution

of the delta of PaO_2/FiO_2 at a specific time point and of PaO_2/FiO_2 at H0 in untreated piglets (SHAM group, N = 12) and acid-injured piglets (HCl group, N = 13). Values are expressed in mmHg and represented as means, with error bars representing standard errors of the means.

Figure 4: Measurements of the alteration in alveolar-capillary membrane permeability. Mini bronchoalveolar levels (BAL) of total protein at 4 h in untreated piglets (SHAM group, N = 12) and acid-injured piglets (HCl group, N = 13). Values are expressed in $g.L^{-1}$ and represented as means, with error bars representing standard errors of the means.

Figure 5: Measurements provided by transpulmonary thermodilution (**A**) Lung edema, as assessed by extravascular lung water. (**B**) Cardiac output. (**C**) Systemic vascular resistance. Transpulmonary thermodilution was performed in untreated piglets (SHAM group, N = 12) and acid-injured piglets (HCl group, N = 13) using a pulse contour cardiac output monitor. Values are expressed in $mL.kg^{-1}$, $L.min^{-1}$, $dynes.s.cm^{-5}$, respectively, and are reported as means, with error bars representing standard errors of the means.

Figure 6: Measurements of the expired fractions of halogenated agents, sevoflurane and isoflurane. (**A**) Expired ($F_E$sevoflurane) and inspired ($F_I$sevoflurane) sevoflurane fractions during the 4-h study period. (**B**) Expired ($F_E$isoflurane) and inspired ($F_I$isoflurane) isoflurane fractions during the 4-h study period. Values are expressed in % and represented as means, with error bars representing standard errors of the means.

Figure 7: Macroscopic evaluation of the whole lung after the 4 h study period. (**A**) Whole lung of an untreated piglet (SHAM group). (**B**) Whole lung of an acid-injured piglet (HCl group). Macroscopic lung injury, with visible hemorrhage and congestion, is noticeable in the red parts of the lung (white arrows). Histologic evaluation of the lung after the 4 h study period**.** (**C**) Histological slice of the lung of an untreated piglet (SHAM group). (**D**) Histological slice of the lung of an acid-injured piglet (HCl group). Histologic evidence of lung injury was a greater cellularity consisting mainly of neutrophils (black arrowheads), with more areas of atelectasis and increased alveolar disruption, hyaline membranes, protein debris, hemorrhage (white arrow), and the thickening of the alveolar wall (black arrows). Scale bars equal 100 µm.

DISCUSSION:

This article describes a reproducible experimental model of ARDS induced by the intratracheal instillation of HCl in piglets to investigate the lung-protective effects of halogenated volatiles, such as sevoflurane or isoflurane, delivered using an anesthetic conserving device.

The primary goal of this study was to develop an experimental model of ARDS in which volatile agents could be delivered by an anesthetic conserving device, such as those used in ICU patients. Although some effects of halogenated agents have been previously studied in animal models, the strength of our model is that it is a clinically relevant, translational model to further our understanding of such effects. Another advantage of this model is that substantial lung injury can be induced in animals larger than mice with low mortality over time. Indeed, an important consideration in the choice of an animal model of ARDS should be the experimental question to be addressed[51]. In a mouse model, experimental techniques to induce lung injuries, such as intravenous oleic acid[52], lavage-induced surfactant depletion[40], and high-stretch mechanical ventilation[53], can induce intensive injury over a timescale from

hours to days, but they do not allow the investigation of lung injury repair/resolution. Furthermore, some challenges of the animal model (e.g., extremely large tidal volumes in certain models of ventilator-induced lung injury) may be extreme, such that they are unrepresentative of the range of conditions present in humans with ARDS. Conversely, such models as intratracheal endotoxin[54] may allow the investigation of certain aspects of the resolution of inflammation and fibrotic processes that can occur following clinical ARDS, but they do not produce the substantial hypoxemia that is a prerequisite for a diagnosis of the syndrome[51]. To characterize their specific effects better, therapeutics should likely be tested in multiple models, as none sufficiently reproduces the heterogeneity of ARDS[55].

Our model has some inherent limitations. First, because we euthanized the animals 4 h after experimental ARDS onset, we only collected parameters during the early phase of ARDS. More elaborate facilities, such as "animal ICUS," are needed to investigate later phases of ARDS in piglets. Second, during the current experiments, we only assessed the degree of lung injury using an index of arterial oxygenation, such as PaO_2/FiO_2. However, most features of experimental ARDS were present when we previously reported use of this acid-induced ARDS model[37]. To improve our model, it could be interesting to add non-invasive measures of the degree of lung injury, determined, for example, using electrical impedance tomography or lung ultrasound[56]. Third, we only report the use of a "one-hit model" to induce lung injury in piglets, while models in which more than one inciting stimulus for lung injury is induced are likely more reflective of the pathological human situation, in which a single inciting stimulus is rarely present ("two-hit hypothesis")[51]. From this perspective, ventilator-induced lung injury could, for example, be added to our model to produce an additional hit, and this model can be combined with other injurious "hits" if needed to investigate more complex clinical scenarios involving multiple features of the ARDS pathophysiology, such as lung endothelial injury, alveolar macrophage activation, and the effects of cell-free hemoglobin[57], among others[58]. Fourth, we did not test other halogenated agents such as desflurane. Indeed, we only used isoflurane and sevoflurane for inhaled sedation in piglets because they are most frequently used in clinical ICU practice, at least in Europe and in some other areas of the world.

The porcine model has contributed to significant advances in experimental research over the last decades, and it has become an increasingly important translational bridge between traditional small laboratory animal models and human medicine. A major advantage of using experimental models in large animals is to allow investigations that involve the ventilation of animals over time. Nevertheless, such models can be extremely expensive, and they may sometimes require the availability of an animal ICU. In addition, the limited availability of some molecular reagents in pigs is an important limitation. Studies in smaller animals, such as mice, rats, or rabbits, have been highly useful in studying individual pathways, but the generalizability of the results to humans appears limited[59]. Larger animal studies can provide focused evaluations of key physiological and molecular pathways and can be used to test new therapies in humans, such as sedation with halogenated agents. Furthermore, the size of the animals supports the use of clinically used catheters, endotracheal tubes, ventilators, and monitors that are not fully available for use in smaller mammals. Indeed, the important advantages of using experimental models with large animals include the ability to take multiple longitudinal blood samples and perform blood gas analyses over time. In addition, invasive hemodynamic monitoring could be used as transpulmonary thermodilution with a

pulse contour cardiac output monitor device, allowing the study of the degree of alveolar edema by measuring extravascular lung water, a highly relevant parameter in the alteration of the alveolar–capillary barrier during ARDS[51]. Nevertheless, caution is necessary when data aside from size are interpreted from animal models, because important anatomical, physiological, and immunological differences exist among animal species. Animal models could have anatomical differences that will impact research and the translation to humans. Indeed, many animals, such as mice or rabbits, have an incomplete mediastinum and thin visceral membranes, prohibiting, for example, the use of the contralateral pleura as a control. However, larger animals (e.g., sheep or pigs) have one pleural cavity around each lung and a thick visceral pleura resembling that of humans[60].

The administration of volatile anesthetics to ICU patients has been increasingly studied in the last decade, mostly because of the development of dedicated devices based either on reflection or on a circle system. Such devices can be inserted into any mechanical ventilation circuit to administer the two agents most frequently used in the ICU setting, sevoflurane and isoflurane[61]. Due to their hypnotic, bronchodilator, and anticonvulsant properties, halogenated agents have been used for a long time in the ICU to manage patients with refractory status asthmaticus, status epilepticus, and complex sedation scenarios with high sedation requirements, such as burns, chronic pain, high-risk surgeries, or a history of drug abuse. Although recent international guidelines do not recommend using volatile agents for procedural pain management[62], halogenated anesthetics are increasingly popular in Europe, and they are considered a feasible option for sedation in the 2015 German Guidelines[63], especially if short wake-up times are needed. Potential therapeutic end-organ-protective properties via the cytoprotective and anti-inflammatory mechanisms[64] of volatile anesthetics have attracted the attention of researchers and physicians. In fact, their emerging use in ICUs has paved the way to the study of their potential benefits in patients with ARDS. ARDS represents the ultimate and most severe form of lung organ dysfunction, as well as a major challenge for patients, their families, healthcare providers from various disciplines, and healthcare systems when caring for critically ill patients, especially under some exceptional circumstances, such as during the current COVID-19 pandemic[65–67]. Beyond the current efforts to find specific antiviral therapies, improving supportive care and treatment options for patients with COVID-19-related ARDS is, therefore, of major importance[65,68,69]. From this perspective, the rationale supporting inhaled sedation with sevoflurane or isoflurane as a way to improve lung epithelial permeability, to decrease the inflammatory response, and, potentially, to improve patient outcomes is strong. In addition, several non-human models have shown that a volatile anesthetic agent, such as inhaled sevoflurane, improves gas exchange[21,70,71], reduces alveolar edema[22], and decreases levels of pro-inflammatory cytokines[72,73]. These effects could be explained by restored lung epithelial function and by the immunomodulatory effects of the halogenated agent. In a previous pilot randomized control trial, the use of a halogenated agent, such as sevoflurane, to sedate ARDS patients in the ICU improved oxygenation and decreased levels of a marker of lung epithelial injury and of some pro-inflammatory cytokines (interleukin [IL]-1β, IL-6, and IL-8 and tumor necrosis factor-α) compared to intravenous sedation[28]. These results reinforce the protective effect of sevoflurane on inflammation and on reduced epithelial injury or improved AFC, as assessed by plasma sRAGE[34].

Understanding the biological mechanisms and pathophysiological pathways involved in acute lung injury and its resolution under inhaled sedation with halogenated agents requires the use of experimental and preclinical models. Although in vitro studies represent an important step in describing these mechanisms[74], in vivo experiments are fundamental before results can be extrapolated to the clinical setting. Furthermore, in this large animal model, halogenated agents could be administered using the same anesthetic conserving device as in humans. In fact, devices based either on reflection or on a circle system, which are both available for patients in some countries, do not have specific equivalents available for small animals, such as for mice, rats, or rabbits. Consequently, when researchers want to administer halogenated agents to animals, they must choose between either pre- or post-exposure to halogenated agents, usually via anesthesia chamber induction over a more or less long time with no specific mechanical ventilation during this period[75]. This piglet model allows the specific reproduction of the same treatment conditions as in ICU patients with ARDS, that is, the administration of halogenated agents, such as sevoflurane, in addition to delivering lung-protective mechanical ventilation with low tidal volumes and PEEP. Interestingly, our model reported the use of the recent, miniaturized version of the anesthetic conserving device to administer sevoflurane for the first time in piglets, thereby allowing smaller tidal volumes and further instrumental dead space to be set compared to the previous version of the device. Furthermore, in addition to administering halogenated volatiles, this model of acid-induced ARDS could be useful in studying specific pathways, such as those involved in lung epithelial injury and its repair[37].

In conclusion, this experimental model of ARDS in piglets has significant advantages compared to the existing ones. These include rapid onset (within 1 h in general), good reproducibility and stability over time, a low mortality rate, and, more importantly, the use of a clinically relevant device to deliver inhaled ICU sedation, thus allowing novel translational approaches to the study of the effects of halogenated agents in ARDS.

ACKNOWLEDGEMENT:
The authors would like to thank the staff from the GreD, the Université Clermont Auvergne, and the Centre International de Chirurgie Endoscopique (all in Clermont-Ferrand, France).

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:

1. ARDS Definition Task Force et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA: The Journal of the American Medical Association*. **307** (23), 2526–2533 (2012).
2. Thompson, B. T., Chambers, R. C., Liu, K. D. Acute Respiratory Distress Syndrome. *The New England Journal of Medicine*. **377** (6), 562–572 (2017).
3. Ware, L. B., Matthay, M. A. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *American Journal of Respiratory and Critical Care Medicine*. **163** (6), 1376–1383 (2001).
4. McAuley, D. F., Frank, J. A., Fang, X., Matthay, M. A. Clinically relevant concentrations of beta2-adrenergic agonists stimulate maximal cyclic adenosine monophosphate-dependent

658 airspace fluid clearance and decrease pulmonary edema in experimental acid-induced lung
659 injury. *Critical Care Medicine*. **32** (7), 1470–1476 (2004).
660 5. Fan, E. et al. An official American thoracic society/European society of intensive care
661 medicine/society of critical care medicine clinical practice guideline: Mechanical ventilation
662 in adult patients with acute respiratory distress syndrome. *American Journal of Respiratory*
663 *and Critical Care Medicine*. **195** (9), 1253–1263 (2017).
664 6. Bellani, G. et al. Epidemiology, patterns of care, and mortality for patients with acute
665 respiratory distress syndrome in intensive care units in 50 countries. *JAMA: The Journal of the*
666 *American Medical Association*. **315** (8), 788–800 (2016).
667 7. De Conno, E. et al. Anesthetic-induced improvement of the inflammatory response to
668 one-lung ventilation. *Anesthesiology*. **110** (6), 1316–1326 (2009).
669 8. Uhlig, C. et al. Effects of volatile anesthetics on mortality and postoperative pulmonary
670 and other complications in patients undergoing surgery: A systematic review and meta-
671 analysis. *Anesthesiology: The Journal of the American Society of Anesthesiologists*. **124** (6),
672 1230–1245 (2016).
673 9. Campagna, J. A., Miller, K. W., Forman, S. A. Mechanisms of actions of inhaled
674 anesthetics. *The New England Journal of Medicine*. **348** (21), 2110–2124 (2003).
675 10. Dikmen, Y., Eminoglu, E., Salihoglu, Z., Demiroluk, S. Pulmonary mechanics during
676 isoflurane, sevoflurane and desflurane anaesthesia. *Anaesthesia*. **58** (8), 745–748 (2003).
677 11. Hert, S. G. D. et al. Choice of primary anesthetic regimen can influence intensive care
678 unit length of stay after coronary surgery with cardiopulmonary bypass. *Anesthesiology*. **101**
679 (1), 9–20, (2004).
680 12. Hashiguchi, H. et al. Isoflurane protects renal function against ischemia and
681 reperfusion through inhibition of protein kinases, JNK and ERK. *Anesthesia and Analgesia*.
682 1584–1589, (2005).
683 13. Fukazawa, K., Lee, H. T. Volatile anesthetics and AKI: risks, mechanisms, and a
684 potential therapeutic window. *Journal of the American Society of Nephrology: JASN*. **25** (5),
685 884–892 (2014).
686 14. Obal, D., Rascher, K., Favoccia, C., Dettwiler, S., Schlack, W. Post-conditioning by a
687 short administration of desflurane reduced renal reperfusion injury after differing of
688 ischaemia times in rats. *British Journal of Anaesthesia*. **97** (6), 783–791 (2006).
689 15. Lv, X. et al. Isoflurane preconditioning at clinically relevant doses induce protective
690 effects of heme oxygenase-1 on hepatic ischemia reperfusion in rats. *BMC Gastroenterology*.
691 **11**, 31 (2011).
692 16. Sakai, H. et al. Isoflurane provides long-term protection against focal cerebral ischemia
693 in the rat. *Anesthesiology*. **106** (1), 92–99 (2007).
694 17. Jerath, A. et al. Volatile-based short-term sedation in cardiac surgical patients: a
695 prospective randomized controlled trial. *Critical Care Medicine*. **43** (5), 1062–1069 (2015).
696 18. Jerath, A., Parotto, M., Wasowicz, M., Ferguson, N. D. Volatile Anesthetics. Is a New
697 Player Emerging in Critical Care Sedation? *American Journal of Respiratory and Critical Care*
698 *Medicine*. **193** (11), 1202–1212 (2016).
699 19. Perbet, S. et al. A pharmacokinetic study of 48-hour sevoflurane inhalation using a
700 disposable delivery system (AnaConDa®) in ICU patients. *Minerva Anestesiologica*. **80** (6),
701 655–665 (2014).
702 20. Mesnil, M. et al. Long-term sedation in intensive care unit: a randomized comparison
703 between inhaled sevoflurane and intravenous propofol or midazolam. *Intensive Care*
704 *Medicine*. **37** (6), 933–941 (2011).

21. Schläpfer, M. et al. Sevoflurane reduces severity of acute lung injury possibly by impairing formation of alveolar oedema. *Clinical and Experimental Immunology*. **168** (1), 125–134 (2012).
22. Voigtsberger, S. et al. Sevoflurane ameliorates gas exchange and attenuates lung damage in experimental lipopolysaccharide-induced lung Injury. *Anesthesiology*. **111** (6), 1238–1248, (2009).
23. Steurer, M. et al. The volatile anaesthetic sevoflurane attenuates lipopolysaccharide-induced injury in alveolar macrophages. *Clinical and Experimental Immunology*. **155** (2), 224–230, (2009).
24. Englert, J. A. et al. Isoflurane Ameliorates Acute Lung Injury by Preserving Epithelial Tight Junction Integrity. *Anesthesiology*. **123** (2), 377–388 (2015).
25. Li, Q. F., Zhu, Y. S., Jiang, H., Xu, H., Sun, Y. Isoflurane preconditioning ameliorates endotoxin-induced acute lung injury and mortality in rats. *Anesthesia and Analgesia*. **109** (5), 1591–1597 (2009).
26. Reutershan, J., Chang, D., Hayes, J. K., Ley, K. Role of a reduction of cytokine levels in isoflurane-mediated protection from endotoxin-induced lung Injury. *Anesthesiology*. **105** (6), 1280–1281, (2006).
27. Du, X. et al. Isoflurane promotes phagocytosis of apoptotic neutrophils through AMPK-mediated ADAM17/Mer signaling. *PloS One*. **12** (7), e0180213 (2017).
28. Jabaudon, M. et al. Sevoflurane for Sedation in Acute Respiratory Distress Syndrome. A Randomized Controlled Pilot Study. *American Journal of Respiratory and Critical Care Medicine*. **195** (6), 792–800 (2017).
29. Yue, T. et al. Postconditioning with a volatile anaesthetic in alveolar epithelial cells in vitro. *The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology*. **31** (1), 118–125 (2008).
30. Blondonnet, R., Constantin, J.-M., Sapin, V., Jabaudon, M. A pathophysiologic approach to biomarkers in acute respiratory distress syndrome. *Disease Markers*. **2016**, 3501373 (2016).
31. Farrell, R., Oomen, G., Carey, P. A technical review of the history, development and performance of the anaesthetic conserving device “AnaConDa” for delivering volatile anaesthetic in intensive and post-operative critical care. *Journal of Clinical Monitoring and Computing*. **32** (4), 595–604, (2018).
32. Sturesson, L. W., Bodelsson, M., Jonson, B., Malmkvist, G. Anaesthetic conserving device AnaConDa: dead space effect and significance for lung protective ventilation. *British Journal of Anaesthesia*. **113** (3), 508–514 (2014).
33. Blondonnet, R. et al. RAGE inhibition reduces acute lung injury in mice. *Scientific Reports*. **7** (1), 7208 (2017).
34. Jabaudon, M. et al. Soluble receptor for advanced glycation end-products predicts impaired alveolar fluid clearance in acute respiratory distress syndrome. *American Journal of Respiratory and Critical Care Medicine*. **192** (2), 191–199 (2015).
35. Jabaudon, M. et al. Soluble forms and ligands of the receptor for advanced glycation end-products in patients with acute respiratory distress syndrome: An observational prospective study. *PloS One*. **10** (8), e0135857 (2015).
36. Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., Altman, D. G. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology*. 8, e1000412 (2010).

751 37. Audard, J. et al. Inhibition of the receptor for advanced glycation end-products in
752 acute respiratory distress syndrome: A randomised laboratory trial in piglets. *Scientific*
753 *Reports*. **9** (1), 9227 (2019).
754 38. Wu, C.-W. et al. Intra-operative neural monitoring of thyroid surgery in a porcine
755 model. *Journal of Visualized Experiments.* (144), e57919 (2019).
756 39. Russ, M. et al. Lavage-induced surfactant depletion in pigs as a model of the acute
757 respiratory distress syndrome (ARDS). *Journal of visualized experiments*. (115), e53610
758 (2016).
759 40. Marumo, C. K. et al. Hemodynamic effects of PEEP in a porcine model of HCl-induced
760 mild acute lung injury. *Acta Anaesthesiologica Scandinavica*. **53** (2), 190–202 (2009).
761 41. Ambrosio, A. M. et al. Effects of positive end-expiratory pressure titration and
762 recruitment maneuver on lung inflammation and hyperinflation in experimental acid
763 aspiration-induced lung injury. *Anesthesiology*. **117** (6), 1322–1334 (2012).
764 42. Sackey, P. V., Martling, C.-R., Granath, F., Radell, P. J. Prolonged isoflurane sedation of
765 intensive care unit patients with the Anesthetic Conserving Device. *Critical Care Medicine*. **32**
766 (11), 2241–2246 (2004).
767 43. Blanchard, F. et al. Minimal alveolar concentration for deep sedation (MAC-DS) in
768 intensive care unit patients sedated with sevoflurane: A physiological study. *Anaesthesia,*
769 *Critical Care & Pain Medicine*. (2020).
770 44. Verghese, G. M., Ware, L. B., Matthay, B. A., Matthay, M. A. Alveolar epithelial fluid
771 transport and the resolution of clinically severe hydrostatic pulmonary edema. *Journal of*
772 *Applied Physiology*. **87** (4), 1301–1312 (1999).
773 45. Sakuma, T. et al. Alveolar fluid clearance in the resected human lung. *American Journal*
774 *of Respiratory and Critical Care Medicine*. **150** (2), 305–310 (1994).
775 46. Matthay, M. A., Wiener-Kronish, J. P. Intact epithelial barrier function is critical for the
776 resolution of alveolar edema in humans. *The American Review of Respiratory Disease*. **142** (6
777 Pt 1), 1250–1257 (1990).
778 47. Ware, L. B., Matthay, M. A. Alveolar fluid clearance is impaired in the majority of
779 patients with acute lung injury and the acute respiratory distress syndrome. *American Journal*
780 *of Respiratory and Critical Care Medicine*. **163** (6), 1376–1383 (2001).
781 48. Ware, L. B., Golden, J. A., Finkbeiner, W. E., Matthay, M. A. Alveolar epithelial fluid
782 transport capacity in reperfusion lung injury after lung transplantation. *American Journal of*
783 *Respiratory and Critical Care Medicine*. **159** (3), 980–988 (1999).
784 49. Constantin, J.-M. et al. Response to recruitment maneuver influences net alveolar fluid
785 clearance in acute respiratory distress syndrome. *Anesthesiology*. **106** (5), 944–951 (2007).
786 50. Kemming, G. I. et al. Effects of perfluorohexan vapor on gas exchange, respiratory
787 mechanics, and lung histology in pigs with lung injury after endotoxin infusion.
788 *Anesthesiology*. **103** (3), 585–594, (2005).
789 51. Matute-Bello, G. et al. An official American Thoracic Society workshop report: features
790 and measurements of experimental acute lung injury in animals. *American Journal of*
791 *Respiratory Cell and Molecular Biology*. **44** (5), 725–738 (2011).
792 52. Chiew, Y. S. et al. Physiological relevance and performance of a minimal lung model:
793 an experimental study in healthy and acute respiratory distress syndrome model piglets. *BMC*
794 *Pulmonary Medicine*. **12**, 59 (2012).
795 53. Hochhausen, N. et al. Optimizing PEEP by Electrical Impedance Tomography in a
796 Porcine Animal Model of ARDS. *Respiratory Care*. **62** (3), 340–349 (2017).

54. Fu, H., Sun, M., Miao, C. Effects of different concentrations of isoflurane pretreatment on respiratory mechanics, oxygenation and hemodynamics in LPS-induced acute respiratory distress syndrome model of juvenile piglets. *Experimental Lung Research*. **41** (8), 415–421 (2015).
55. Yehya, N. Lessons learned in acute respiratory distress syndrome from the animal laboratory. *Annals of Translational Medicine*. **7** (19), 503 (2019).
56. Hochhausen, N. et al. Comparison of two experimental ARDS models in pigs using electrical impedance tomography. *PloS One*. **14** (11), e0225218 (2019).
57. Shaver, C. M. et al. Cell-free hemoglobin: a novel mediator of acute lung injury. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. **310** (6), L532–541 (2016).
58. Matthay, M. A. et al. Acute respiratory distress syndrome. *Nature Reviews. Disease Primers*. **5** (1), 18 (2019).
59. Martin, T. R., Matute-Bello, G. Experimental models and emerging hypotheses for acute lung injury. *Critical Care Clinics*. **27** (3), 735–752 (2011).
60. Light, R. W., Gary Lee, Y. C. *Textbook of Pleural Diseases Second Edition*. CRC Press. (2008).
61. Laferriere-Langlois, P., d'ARAGON, F., Manzanares, W. Halogenated volatile anesthetics in the intensive care unit: current knowledge on an upcoming practice. *Minerva Anestesiologica*. **83** (7), 737–748 (2017).
62. Devlin, J. W. et al. Clinical Practice Guidelines for the Prevention and Management of Pain, Agitation/Sedation, Delirium, Immobility, and Sleep Disruption in Adult Patients in the ICU. *Critical Care Medicine*. **46** (9), e825–e873 (2018).
63. DAS-Taskforce 2015 et al. Evidence and consensus based guideline for the management of delirium, analgesia, and sedation in intensive care medicine. Revision 2015 (DAS-Guideline 2015) - short version. *German Medical Science: GMS e-journal*. **13**, Doc19 (2015).
64. O'Gara, B., Talmor, D. Lung protective properties of the volatile anesthetics. *Intensive Care Medicine*. **42** (9), 1487–1489 (2016).
65. Murthy, S., Gomersall, C. D., Fowler, R. A. Care for critically ill patients with COVID-19. *JAMA: The Journal of the American Medical Association*. (2020).
66. Liao, X., Wang, B., Kang, Y. Novel coronavirus infection during the 2019-2020 epidemic: preparing intensive care units-the experience in Sichuan Province, China. *Intensive Care Medicine*. **46** (2), 357–360 (2020).
67. Grasselli, G., Pesenti, A., Cecconi, M. Critical care utilization for the COVID-19 outbreak in Lombardy, Italy: Early experience and forecast during an emergency response. *JAMA: The Journal of the American Medical Association*. (2020).
68. Arentz, M. et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington State. *JAMA: The Journal of the American Medical Association*. (2020).
69. Xu, Z. et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet. Respiratory Medicine*. **8**(4), 420-422 (2020).
70. Ferrando, C. et al. Sevoflurane, but not propofol, reduces the lung inflammatory response and improves oxygenation in an acute respiratory distress syndrome model: a randomised laboratory study. *European Journal of Anaesthesiology*. **30** (8), 455–463 (2013).
71. Voigtsberger, S. *et al.* Sevoflurane ameliorates gas exchange and attenuates lung damage in experimental lipopolysaccharide-induced lung injury. *Anesthesiology*. **111** (6), 1238–1248 (2009).

72. Suter, D. et al. The immunomodulatory effect of sevoflurane in endotoxin-injured alveolar epithelial cells. *Anesthesia and Analgesia*. **104** (3), 638–645 (2007).
73. Steurer, M. et al. The volatile anaesthetic sevoflurane attenuates lipopolysaccharide-induced injury in alveolar macrophages. *Clinical and Experimental Immunology*. **155** (2), 224–230 (2009).
74. Blondonnet, R. et al. In vitro method to control concentrations of halogenated gases in cultured alveolar epithelial cells. *Journal of Visualized Experiments.* (144), e58554 (2018).
75. Gargiulo, S. et al. Mice anesthesia, analgesia, and care, Part I: Anesthetic considerations in preclinical research. *ILAR Journal / National Research Council, Institute of Laboratory Animal Resources*. **53** (1), E55–69 (2012).

Figure1

Click here to access/download;Figure;Copie de Copie de Figure1_JOVE2020.jpg

Figure2

Click here to access/download;Figure;Copie de Copie de Figure2_JOVE2020.jpg

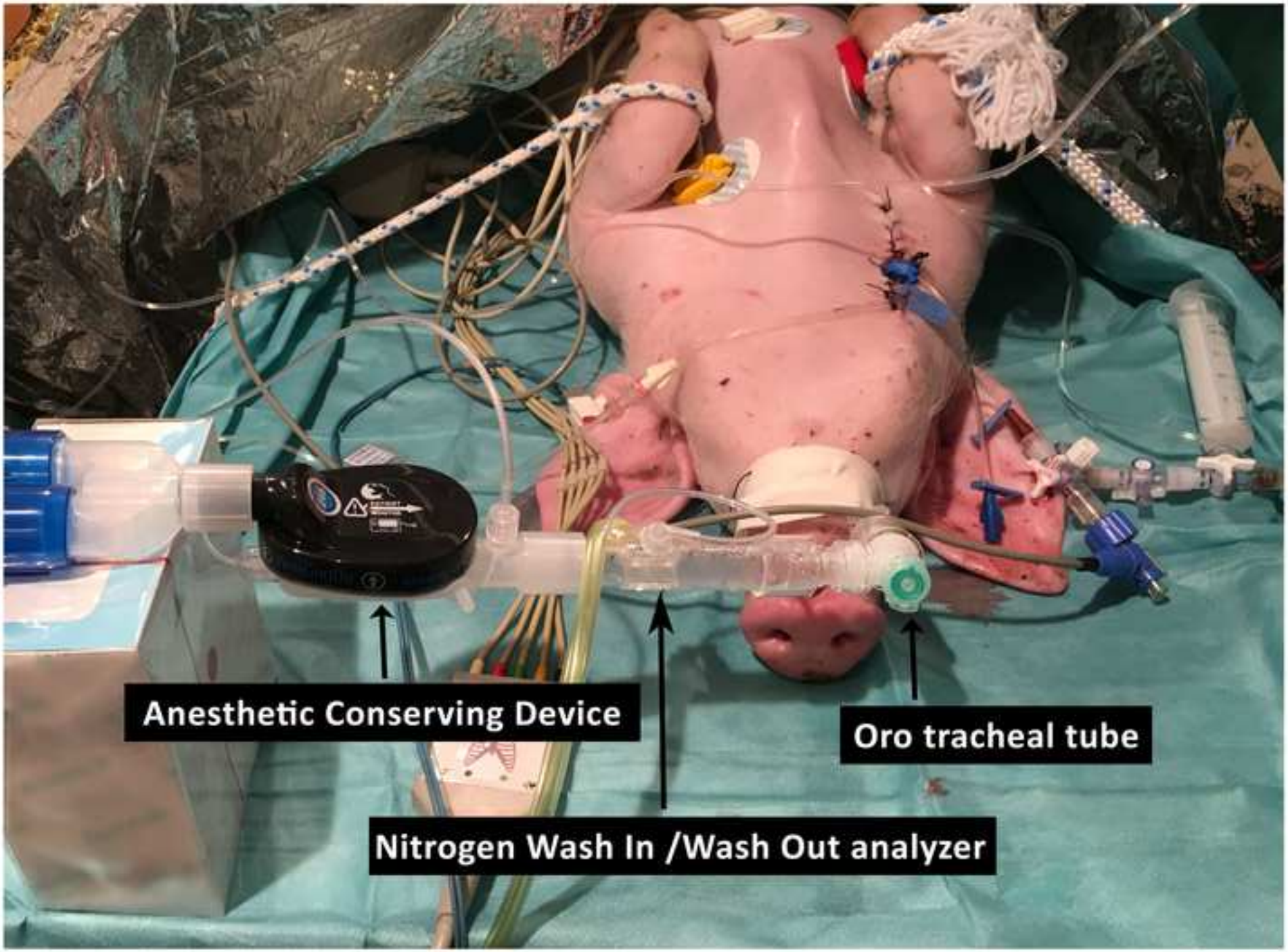


Figure3

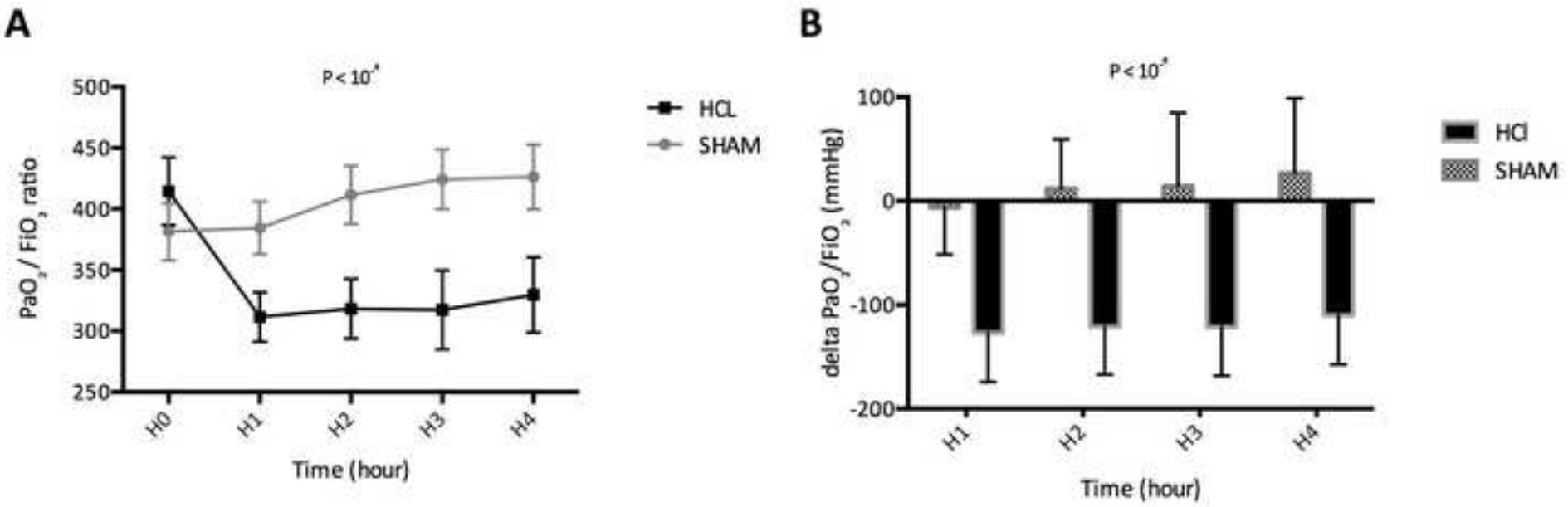


Figure4

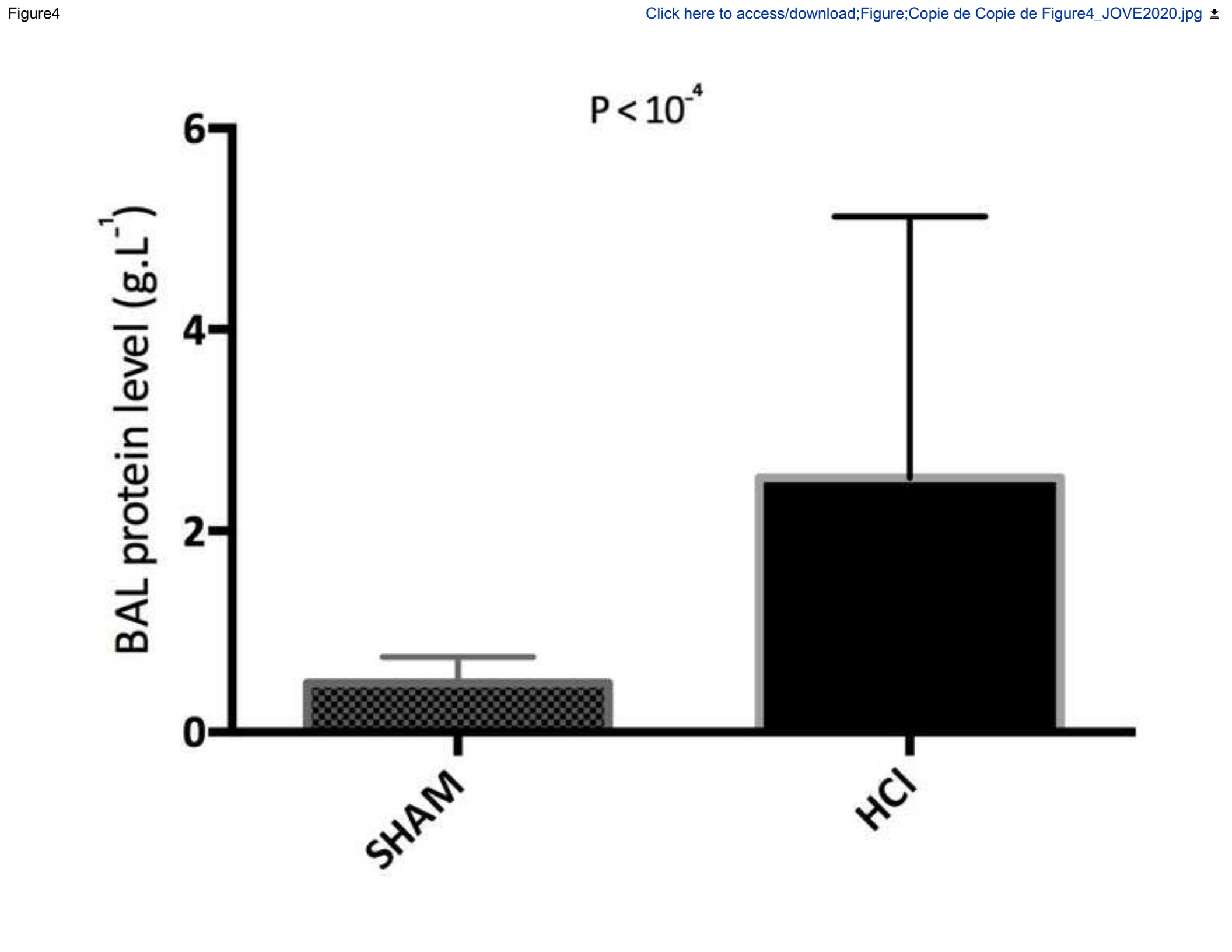


Figure5

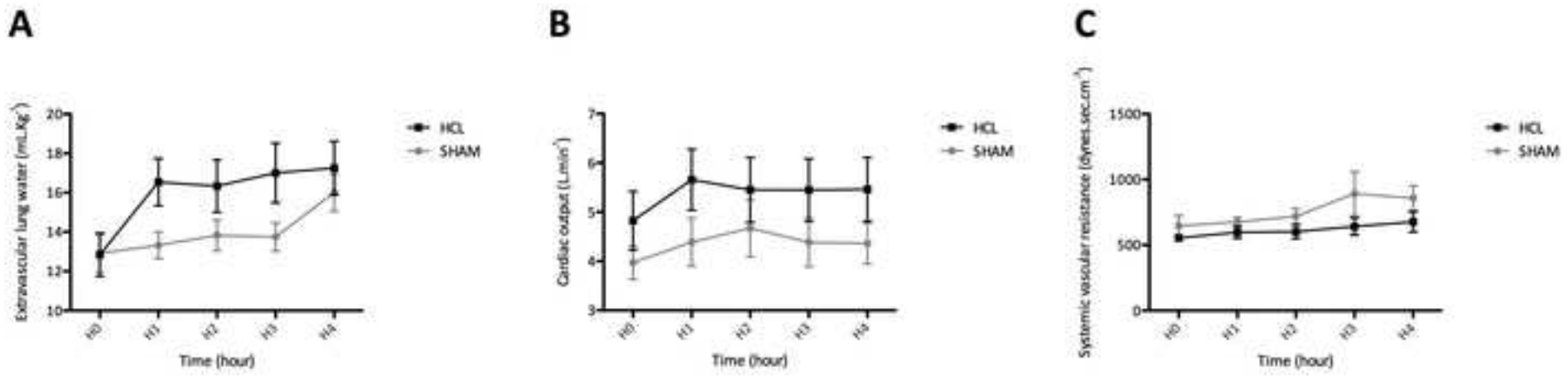


Figure6

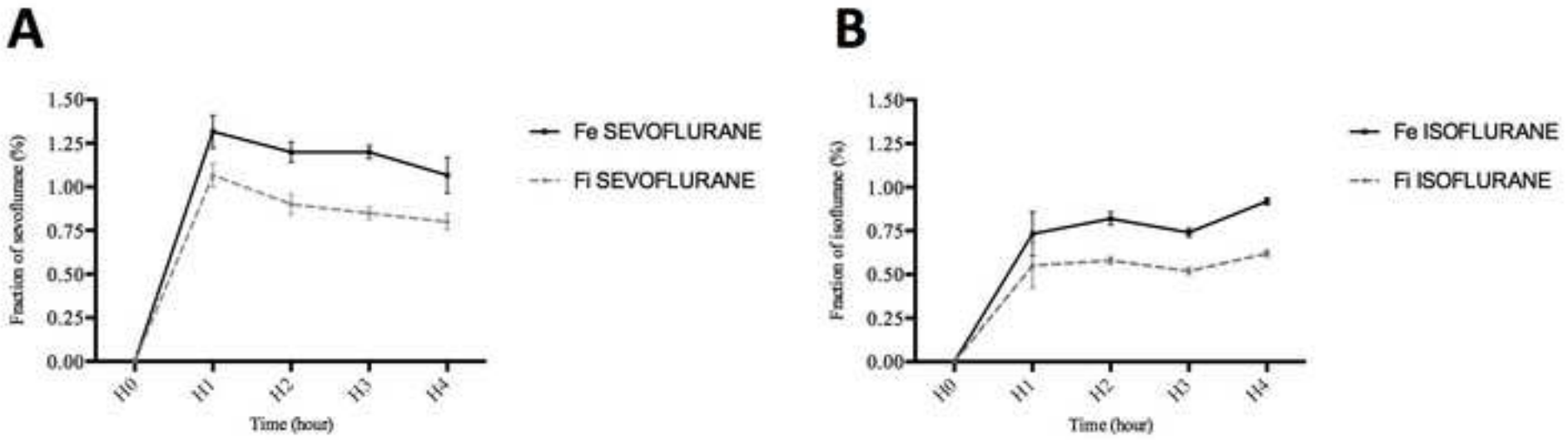
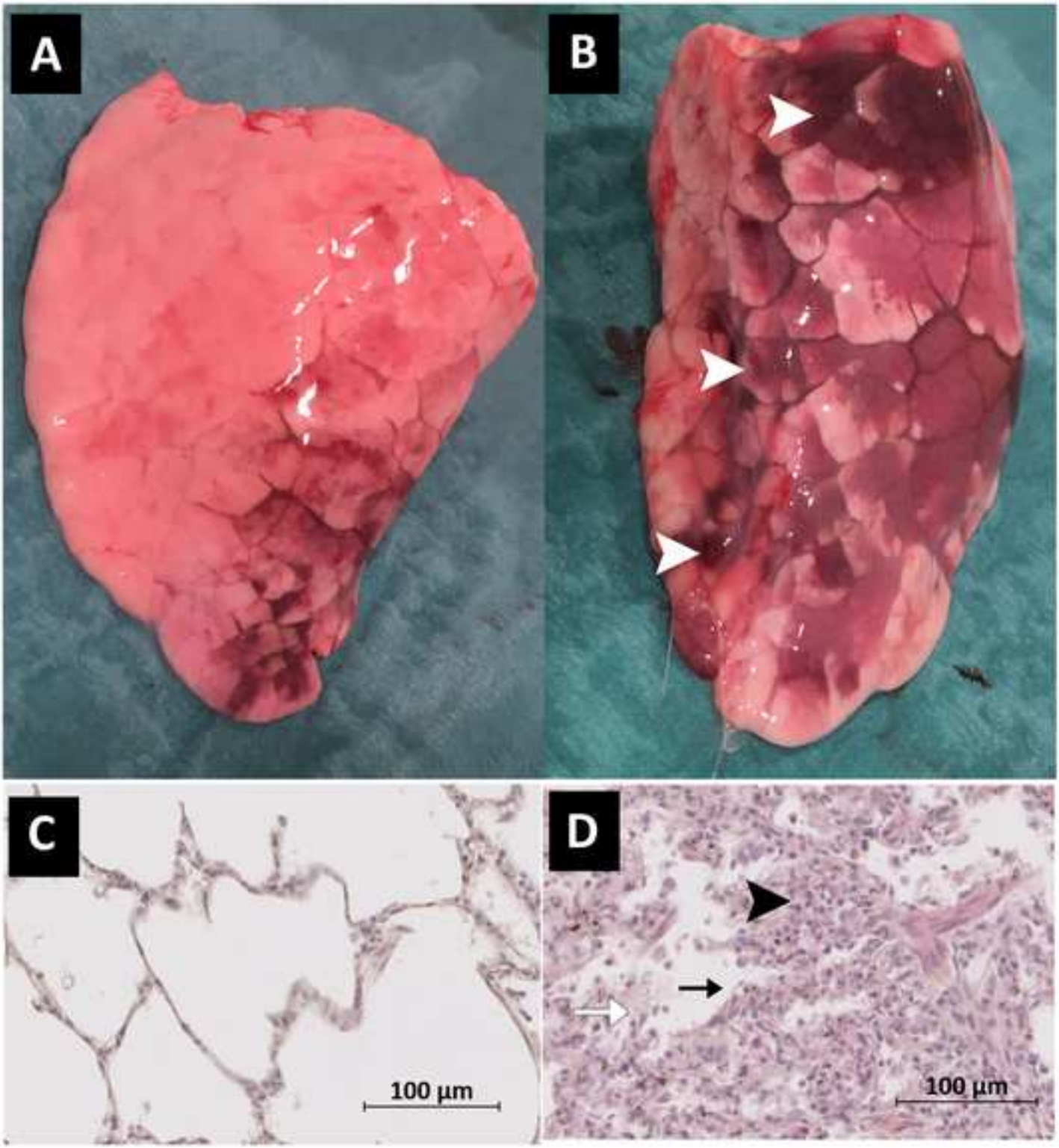


Figure7

Click here to access/download;Figure;Copie de Copie de Figure7_JOVE2020.jpg



Name of Material/ Equipment

Tracheal intubation
Endotracheal tube 6-mm

Animal preparation
Central venous catheter 3-lumens catheter (7 French - 16 cm)
Pulse contour cardiac output monitor PiCCO catheter (3-5 French - 20 cm)
Warm blankets WarmTouch5300

Monitoring
External monitor IntelliVue MP40
Point-of-care blood gas analyzer Epoc® Blood Analysis System
Pulse contour cardiac output monitor PiCCO Device PulsioFlex Monitor

Mechanical ventilation
Ventilator Engström Carestation

Halogenated anesthetics
Anaconda Syringe
Anesthetic conserving device AnaConDa-S
Charcoal filter FlurAbsorb
Filling Adaptaters
Ionomer membrane dryer line Nafion

Products
Propofol
Isoflurane
Pentobarbital
Sevoflurane
Sufentanil

Company	Catalog Number	Comments/Description
Covidien	18860	
Arrow	CV-12703	
Getinge Pulsion Medical System	catheter	
MedTronic	5300	
Phillips	MNT 142	
Siemens	20093	
Getinge Pulsion Medical System	PulsioFlex	
General Electrics	Engström	
SedanaMedical	26022	
SedanaMedical	26050	
SedanaMedical	26096	
SedanaMedical	26042	
SedanaMedical	26053	
Mylan	66617123	
Virbac	QN01AB06	
PanPharma	68942457	
Abbvie	N01AB08	
Mylan	62404996	

RESPONSE TO EDITOR AND REVIEWER COMMENTS

Manuscript ID: JoVE61644

Title: **Effects of halogenated agents delivered through an intensive care unit-type device in a porcine model of acute respiratory distress syndrome**

Authors: Raiko Blondonnet[1,2], Bertille Paquette[1,2], Jules Audard[1,2], Ridvan Guler[1,2], François-Xavier Roman[1,2], Ruoyang Zhai[2], Corinne Belville[2], Loïc Blanchon[2], Thomas Godet[1], Emmanuel Futier[1,2], Jean-Etienne Bazin[1], Jean-Michel Constantin[3], Vincent Sapin[2,4], Matthieu Jabaudon[1,2,5]

We thank the Editor and the Reviewers for their careful read and thoughtful comments on the previous version of our manuscript. We have carefully taken their comments into consideration in preparing our revision, and we hope the manuscript has been improved. Please find below a point-by-point response to the comments and questions.

COMMENTS FROM THE EDITOR

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

As recommended, we have carefully proofread our revised manuscript and we hope there will not be any spelling and grammatical errors left.

• **Textual Overlap:** Significant portions of the manuscript show significant overlap with previously published work. Please re-write the text highlighted in the attached PDF report document within the introduction and discussion using ALL original text to avoid this overlap.

In our revision, we have thoroughly tracked any kind of overlap with previous work, and have made some minor changes in the Introduction and Discussion sections, as indicated.

However, it seems that many other parts of the "text overlap" verification process that has been performed spotted some very generic expressions or sentences that we will not be able to change (for example: *"alveolar inflammation and epithelial injury during ARDS", "in a large animal model", "Ethics committee of the French "Ministere de l'Education Nationale, de*

l'Enseignement Superieur et de la Recherche"", "Centre International de Chirurgie Endoscopique, Universite Clermont Auvergne, Clermont-Ferrand, France", etc.).

• **Protocol Highlight:** Add a one line space after each step. After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

As recommended, we have added a one-line space after each experimental step. We also provided the Editor with an additional document that highlights in yellow the filmable content *per se*.

• Define error bars in all figures.

Thank you. In our revised manuscript, we have now defined all error bars (standard errors of the means) in all figure legends. Figure 6 has also been rearranged to better visualize these error bars.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We agree with this important comment from the Editor. Under its previous form, the Discussion was rather long and unfocused. We have revised this section accordingly and hope it will read better now.

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are (AnaConDa, Sedana Medical, AnaConDa, (AnaConDa-S, Miller, WarmTouch, Medtronic, (IntelliVue MP40, Phillips, Seldinger, PiCCO device (Pulsion SA, (Ventilator Engström Carestation, GE Healthcare, (FlurAbsorb - Sedana Medical, Nafion, BD Preset, Epoc® Blood Analysis System, Siemens, Inview™ (ConvaTec, BD Luer-Lok, etc.

1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

We apologize for including these brands in our previous manuscript.

As requested, we have deleted all commercial sounding elements from the main manuscript and have referred to the table of materials/reagents for further details and specific commercial names, if any.

• **Table of Materials:** Sort the list alphabetically.

In our revision, we have reorganized the list from the Table of Materials has been re-organized alphabetically. Thank you.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

All figures and tables are original and have never been published previously.

COMMENTS FROM THE REVIEWERS

REVIEWER #1:

Manuscript Summary:

The manuscript is very well written and describes in great detail the procedures required for this porcine model of HCL induced lung injury.

Major Concerns:

I have no major concerns with this manuscript

We thank Reviewer #1 for his/her kind and positive comments.

Minor Concerns:

If the authors desire, they could address the following before submission. However, this in my opinion is not required prior to publication.

1) Use of the Anaconda

- The authors describe mainly a protocol of experimental porcine induced lung injury via HCl while administering volatile agents in the post exposure period. Although it is clearly matched to the investigators' clinical research with volatile anesthetic sedation via the Anaconda, it is not clear why the device needs to be used during this experiment. If the anesthetic is to be administered after endotracheal intubation, then it should be possible to use a conventional vaporizer or anesthesia machine to administer the agent. For investigators who may choose to replicate this model in future studies, perhaps without the anaconda, this possibility could be acknowledged. If the Anaconda is preferable over a conventional system, the authors should state why this would be the case

We thank Reviewer #1 for this important comment and understand his/her concern. As a matter of fact, more conventional anesthesia setups could be easily used to replicate this model.

However, our specific goal here was to develop a model in which we could induce ARDS in animals and treat them with inhaled isoflurane or sevoflurane in a manner that would match as much as possible the clinical situation of inhaled ICU sedation, i.e. through a dedicated device different from anesthesia ventilators.

In this specific setting of inhaled ICU sedation, and to the best of our knowledge, only two devices are available for clinical use: the AnaConDa (Sedana Medical) and the Mirus (MedCaptain by Anandic Medical Systems); however, the choice of the miniaturized AnaConDa-S was largely motivated by its lower instrumental deadspace volume, compared to the Mirus.

In the revised version of the manuscript, we have added these justifications and unique features of our model in order to better explain this choice.

2) sRAGE

- The assay currently does not include sRAGE. Is this because this biomarker is not available in pigs? The investigators' primary work in this area involves sRAGE, therefore it would likely be beneficial to include this biomarker in their model.

We thank Reviewer #1 for this comment. As mentioned, our team is specifically interested in the roles of the RAGE pathway in the mechanisms of acute lung injury and repair, and in the value of soluble RAGE as a biomarker of lung epithelial injury.

As a matter of fact, we have multiple translational projects ongoing, or just about to start, to test the hypothesis that at least some of the pulmonary effects of inhaled halogenated agents might be mediated by the RAGE pathway. However, this is out of the scope of the current submission, the latter being specifically focused on a clinically-relevant method to deliver inhaled sedation in large animals with ARDS, such as we would do in patients admitted to an intensive care unit.

3) 2 hit model

- It would be fairly simple to include VILI in their 2-hit models of lung injury in the future. This could be acknowledged

We again thank Reviewer #1 for this very smart comment. Unfortunately, we did not include such a second-hit (eg, VILI) in our current experimental model. As suggested, we have added a sentence in the Discussion to acknowledge this point.

4) Stability and low mortality of the model

- Although the authors state these characteristics as positive attributes of the model, there is no information available in the text or figures to back up this assertion.

Thank you very much for this important remark. This was a mistake in our previous manuscript and we have now added details on mortality data in the revised Results section.

Indeed, no fatal course was observed when we used the model as currently described.

REVIEWER #2:

Manuscript Summary:

The authors present comprehensively an animal model of hydrochloric acid-induced acute respiratory distress syndrome (ARDS) in piglets. These pigs received in addition anesthesia by inhalation with halogenated agents isoflurane and sevoflurane (but why not desflurane?). This model should be used to investigate the biological mechanisms of the potential protective effects of halogenated agents during lung injury and repair, but it is insufficiently performed.

We thank Reviewer #2 for his/her comments.

In this model, we "only" used isoflurane and sevoflurane for inhaled sedation in piglets because these are the two halogenated agents that are the most used in clinical practice, at least in Europe and in some other areas of the world.

The use of desflurane for inhaled ICU sedation is more challenging (it is only available through one device, the Mirus, because its boiling point, between 23°C and 24°C, is close to room temperature) and is associated with a much higher cost compared to isoflurane and sevoflurane. In addition, there are important concerns regarding atmospheric pollution with desflurane, to the extent that it is less and less used for general anesthesia in the operating room.

This has been added as a piece of discussion in our revised manuscript.

Major Concerns:

My first and most important comment is, that the effects of halogenated agents in the lungs are well-known for decades and these have been systematically examined (please compare to the studies by Kotani et al., already in the late nineties!). In addition, there was established a plethora of models of lung injury including VILI and different models ARDS (acid, mechanical forces, lavage; please compare to the group of Hedenstierna et al.) that has been already used in the last century.

The authors state that they developed an optimised piglet model of acid aspiration; however, I am rather reluctant to that claim. In detail, the authors failed to explain what the unique features are of their present model.

Accordingly, they shouldn't write what they already know about ARDS, they should better provide evidence regarding the outstanding aspects of their animal model. Where are the "optimized features"?

We fully agree with this analysis from Reviewer #2.

Many experimental models of ARDS have been used and published to date, including this model of intratracheal hydrochloric acid-induced lung injury in piglets (Audard et al. Scientific Reports 2019. doi: 10.1038/s41598-019-45798-5).

However, the specificity of our current protocol relies on the use of a specific system to deliver inhaled sedation, that is already available to intensive care providers and is therefore very clinically relevant.

In this perspective, the use of the Anaesthesia Conserving Device (AnaConDa, Sedana Medical), along with a strategy of lung-protective mechanical ventilation, also echoes perfectly the ongoing clinical studies of inhaled sedation such as our multicenter randomized trial (SESAR; https://clinicaltrials.gov/ct2/show/NCT04235608), among others.

We did our best to better highlight this specificity of our experimental model throughout the manuscript, and we removed the term "optimised" from the revised text.

In addition, I have major methodological concerns:

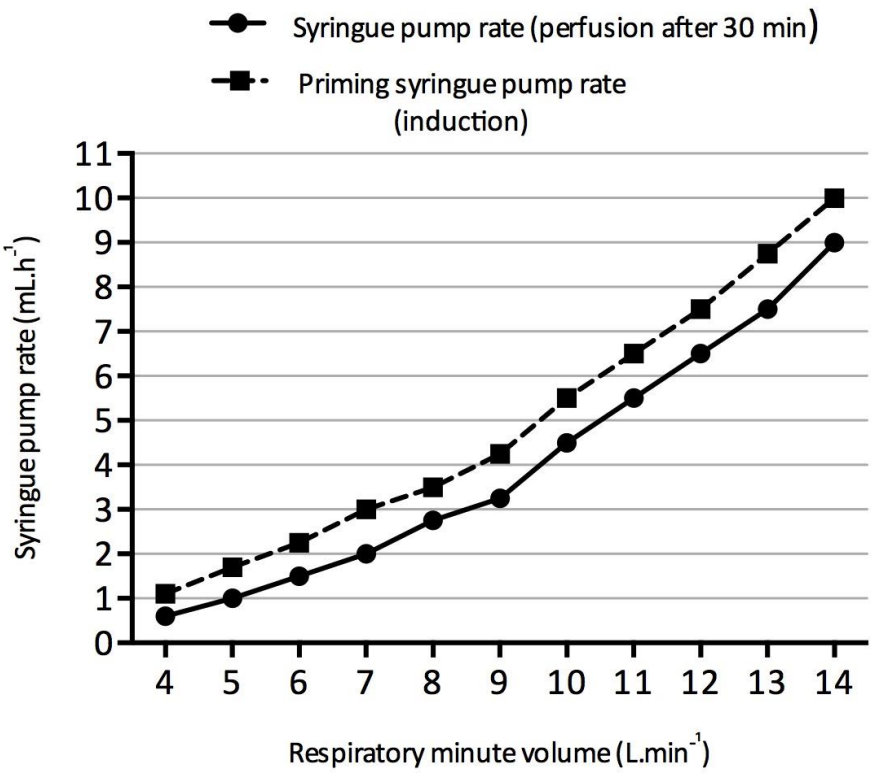
-For what reasons the authors did abstain from a tracheostomy: It is much more suitable in piglets, easy to perform, and the data are more reliable.

We thank Reviewer 2 for this interesting comment. Although we agree with Reviewer #2 that tracheostomy can be performed rather easily in piglets, our team has extensive experience and expertize in orotracheal intubation of animals and we are confident that our data are as reliable as if they were obtained using a tracheostomy. In addition, we believe the use of orotracheal intubation, which is the most frequent situation in the clinical setting of patients with ARDS, increases the translational value of our model, especially with regards to instrumental deadspace of the respiratory circuit.

-How was sufficient deep of anesthesia ensured?

We thank Reviewer #2 for this pivotal question and the opportunity we are given to better explain how the level of sedation was set in our model.

Here, the initial infusion rate of sevoflurane was set based on the instructions from the AnaConDa-S manufacturer, i.e. based on an algorithm considering minute ventilation, as reported in the following graph:



Then, the infusion rate of sevoflurane was titrated to reach a target of expired sevoflurane fraction ($F_E$sevo, which is a reliable surrogate for cerebral concentrations of sevoflurane in patients) of 0.8-1.1. In a recent publication from our team (doi: 10.1016/j.accpm.2020.04.002), $F_E$sevo values have recently been associated to the minimal alveolar concentration (MAC) of sevoflurane and, more importantly, to the Richmond Assessment Sedation Score (RASS), as the RASS is used routinely in most ICUs to monitor and titrate sedation. Briefly, in this clinical study, such $F_E$sevo (of 0.8–1.1) were associated with deep sedation levels in ≥95% of ICU patients.

We have added a sentence to better explain this point to the readers in the Discussion section of the revised manuscript.

-An ID 6mm tube is too small for sufficient measurements and interventions.

We thank Reviewer #2 for this comment, although we partially disagree with this assertion.

We believe that a 6 mm-ID tube is very adequate to use in 10-15 kg-piglets and to deliver mechanical ventilation in safe conditions, without, for example, increasing the resistance of the airways. As a matter of fact, this is what we have always been using in such animals (see Audard et al. Scientific Reports 2019. doi: 10.1038/s41598-019-45798-5). In particular, a 6 mm-ID tube allows insertion of a CH14 suction catheter (such as to perform the intracheal aid

instillation or to sample pulmonary fluid). In addition, for these experiments, we used a modern ICU ventilator that easily allows the delivery of volume-controlled ventilation with such a 6 mm-ID tube.

-What is the difference to the widely used oleic-acid induced ALI model?

We thank Reviewer #2 for this question. We did not choose the oleic-acid model in our study for two reasons.

First, we chose the hydrochloric acid model because it is described as a better model of direct lung epithelial injury, which is the main topic of our research team. In contrast, the oleic acid model has often been described as a model that mimics ARDS due to fat embolism, with only little degree of alveoalr inflammation and epithelial injury; oleic acid can however induce overwhelming lung injury, but on a timescale that is very variable (from hours to days). As it does not really allow the investigation of mechanisms of lung repair and ARDS resolution (in general), we rather opted for the hydrochloric acid model as some of projects will also include the study of the later phases of fibroproliferation (such as in doi: 10.1183/09031936.00093911).

Second, hydrochloric acid-induced ARDS is, in general, associated with minimal hemodynamic compromise compared to other models (which could explain the low mortality rate), especially when injury is at its peak.

We have added a new section to the revised Discussion to better explain this choice.

-There was CV and arterial lines in the pigs; however, for this model a PAC and a PiCCo catheter are urgently needed. Without Qs/Qt, SVR/PVR, CO/CI and DO2 and VO2 calculations/ measurements the model is worthless.

Actually, we used a PiCCO system in our experiments, as described in the manuscript. We only chose to report PiCCO-derived measurements of lung edema in our model (see Figure 5 with extravascular lung water) as we thought it would best illustrate this major feature of experimental ARDS (namey alveolar-capillary permeability).

Because we also recorded the CI and SVR, we have added them to Figure 5. However, we regret we did not collect data that are required for VO2 calculation.

-In my experience, anesthesia in pigs with remifentanil (and cisatracurium!!!) is insufficient, the authors used the 100-fold dosage as compared to humans resulting in significant bias!!!

We thank Reviewer #2 for this comment. However, we are afraid there may be an important misunderstanding here.

As a matter of fact, piglets were of course not anesthetized with remifentanil and cisatracurium alone. During the first phase of our model (before lung injury is induced), piglets were under intravenous sedation with propofol, in addition to remifentanil-based analgesia and neuromuscular blockade with cisatracurium. During the second phase, the propofol was stopped and sedation switched to inhaled sedation with the halogenated agent (sevoflurane or isoflurane).

About the remifentanil doses we used, they are those recommended by the French Medicine Agency (Agence Nationale de la Sécurité du Médicament, ANSM) for pain management during general anesthesia in patients (0.25 $\mu g.kg^{-1}.min^{-1}$ (= 15 $\mu g.kg^{-1}.h^{-1}$). In addition, such doses are usually being used in piglets when combined to a hypnotic agent (see doi: 10.1186/s13028-016-0223-6).

We did our best to clarify this point in the revised manuscript by writing the two units, in order to avoid the risk of misunderstanding by the readers.

-BAL itself induces additional severe alveolar injury and must be therefore avoided in baseline/standard measurements!

We agree that performing a BAL could in itself induce a certain degree of lung injury. However, here we used a mini-BAL technique (with a maximum of 50 mL of NaCl 0.9%), which is far below the volume usually associated with acute lung injury due to surfactant depletion. In addition, BAL to measure the concentration in total protein and/or in a high molecular weight protein is considered as a very relevant parameter in order to measure the alteration of the alveolar-capillary barrier (*Official American Thoracic Society Workshop Report: Features and Measurements of Experimental Acute Lung Injury in Animals*. doi: 10.1165/rcmb.2009-0210ST). Furthermore, in order to limit lung effects of the instillation of saline, in our model mini-BAL was performed 4h after the acid instillation i.e., at the end of the experiment and just before the euthanasia of the piglet.

Accordingly, we have highlighted in our revision that a mini-BAL was performed in our model (as opposed to a more conventional BAL).

Taken together the present model delivers nothing useful. It exerts no advantages in comparison with the lung injury models that we have established in the last 30 years. In addition, the manuscript lacks from important data and facts and is poorly written.

This manuscript will not reach scientific priority for publication.

Although we can understand this point of view, we strongly disagree with these statements. As explained above, the strengths of our experimental model are not to describe a new model for ARDS in piglets but to deliver inhaled sedation to these injured animals using a highly clinically-relevant method that is also used in ICU patients.

REVIEWER #3:

Manuscript Summary:

The study by Blondonnet et al. demonstrated an acid induced ARDS disease model using a piglets. The model demonstrated significant difference in PaO2/FiO2 ratio, BALF protein, extravascular lung water and the efficacy of anesthetic delivery device in maintaining the anesthesia levels after induction. HCL induced acute lung injury models in piglets are already well established. However, this model shows efficacy relates to lung injury induced by HCL. Measurable differences in various lung injury parameters are statistically different. Anesthetics such as isoflurane and sevoflurane are known to ameliorate LPS-mediated or other forms of acute lung inflammation and injury. In this regard, this piglet

Major Concerns: None

Minor Concerns: None

We sincerely thank Reviewer 3 for his/her kind and positive comments.