Online Supplemental Material

Sheep Preparation, Instrumentation and Measurements

Neither systemic nor topical antibiotics were administered to any sheep in the four weeks prior to the study and during the experiment. Sheep were anesthetized (loading dose of 7 mg·kg⁻¹ ketamine and maintenance with continuous infusion of ketamine between 22–36 mg·kg⁻¹·hr⁻¹), orotracheally intubated with a standard 8-mm ETT and connected to a mechanical ventilator (Servo 900C; Siemens Elema, Solna, Sweden). During the surgical preparation sheep were ventilated in pressure support (PS) (PS level 5 cm H₂O, positive end-expiratory pressure (PEEP) 5 cmH₂O, and FiO₂ 0.4) with the ETT cuff inflated to a pressure of 40 cm H₂O. The ventilator circuit was composed of low-compliance silicone rubber tubing and was 100% humidified through a heated (37.5°C) respiratory humidifier (MR850 JHU; Fisher & Paykel, Auckland, New Zealand). All the circuits used for the ventilator were sterile and equally managed in the two groups.

We first aspirated via a nasogastric tube the gastric content with the rumen of the sheep, to reduce intra-abdominal pressure when the animals were in the supine position. Subsequently, we performed the surgical insertion of vascular catheters, under strict asepsis and direct vision.

We cannulated:

1. the carotid artery, for systemic arterial pressure monitoring (Hewlett-Packard M117 66 monitor, Palo Alto, CA) and collection of blood samples for analysis by using an Anova Statplus 9 blood gas analyzer (Nova Biomedical, Waltham, MA);
and 2. the pulmonary artery, with a 7 Fr, four-lumen pulmonary artery catheter (Abbott Critical Care Systems, Chicago, IL) through the right external jugular vein, to measure pulmonary artery pressure, central venous pressure, pulmonary artery occlusion pressure, core blood temperature and cardiac output by thermodilution (9520A Cardiac Output Computer; American Edwards Laboratories, Irvine, CA).

Following surgery, sheep were placed in the prone position, for all the study, with ETT cuff pressure maintained at 25 cm H$_2$O by using a water bottle. We introduced an n. 12 Foley catheter into the bladder to monitor urinary output. We needed, on the average, 4h for induction of anesthesia, surgical preparation and establishing baseline values.

After this preliminary phase, ketamine infusion was discontinued and we maintained anesthesia through a continuous infusion of sodium pentobarbital at a rate of 8 mg·kg$^{-1}$·hr$^{-1}$. At this point, pancuronium bromide was used to paralyze sheep (loading dose 0.15 mg·kg$^{-1}$, maintenance dose of 0.08 mg·kg$^{-1}$·hr$^{-1}$).

Ventilation was switched in volume-controlled, at a tidal volume of 8–10 mL·kg$^{-1}$, respiratory rate 14–25 breaths·min$^{-1}$, PEEP 4–5 cm H$_2$O, and FiO$_2$ 0.4. We adjusted ventilator settings during the experiment in order to keep PaCO$_2$ between 35 and 45 mm Hg and a peak pressure <20 cm H$_2$O. Liquid infusion was set at 6.5 mL·kg$^{-1}$·hr$^{-1}$ of 1:1 of Ringer's lactate and 0.9% NaCl in all sheep. The feeding of the distal gut was provided without use of prokinetic drugs. The enteral nutrition was composed of 350 g/day dry
grass/grain pellets dispersed in 300 mL of water (three or four portions per day) using an 18-Fr nasogastric tube (Bard Levin Tube, C.R. Bard, Covington, GA).

Arterial blood gases, hemodynamic status, respiratory function, ventilator settings, body temperature and water balance were hourly monitored. We checked blood counts and performed chest x-rays: after preparation (baseline) and after 24h of mechanical ventilation before extubation in both the two groups, and at 48h after the extubation in the awaken group. Toilettre of the mouth and of the perineal area of the animals was carefully provided. Iodine solution was applied daily to surgical incisions, but no topical antibiotics were used.

After extubation, sheep that had been awakened were placed in the NIH stables where the animals had free access to water and food. The animals were clinically re-evaluated every 12h. Clinical assessment along with arterial blood gas analysis and blood chemistry was performed at 24h after mechanical ventilation (both groups) and at 48h after extubation (Awake Group) until the end of the experiment. Both groups were euthanized with an intravenous injection of a high dose of sodium pentobarbital and 60 mEq of potassium chloride.