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Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats

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ABSTRACT

General anesthesia is frequently associated to transient hypoxemia and lung atelectasis. Although volatile anesthetics are safe and widely used, their potential role on anesthesia-induced pulmonary impairment has not been fully explored. In this study, we investigated the effect of volatile anesthetic sevoflurane on pulmonary surfactant composition and structure that could contribute to atelectasis. After 30 min of sevoflurane anesthesia, Sprague–Dawley rats showed increased levels of lyso-phosphatidylcholine and decreased levels of phosphatidylcholine associated with significant impairment in lung mechanics and alveolar collapse, but showed no deterioration of alveolar fluid reabsorption when compared to control group of rats anesthetized with pentobarbital. Exposure to sevoflurane altered the thermotropic profile of surfactant model membranes, as detected by fluorescence anisotropy. In this sense, sevoflurane-promoted fluidification of condensed phases could potentially impair the ability of surfactant films to sustain the lowest surface tensions.

In conclusion, the observed changes in surfactant composition and viscosity properties suggest a direct effect of sevoflurane on surfactant function, a factor potentially involved in anesthetic-induced alterations in lung mechanics.

1. Introduction

More than a century ago Meyer [1] and Overton [2] proposed a strong correlation between the potency of anesthetics and their solubility in olive oil. Despite its elegance, certain exceptions to the Meyer–Overton correlation apply to volatile anesthetics based on their interaction with lipid bilayers [3]. Although volatile anesthetics are safe and widely used in general anesthesia, pulmonary surfactant and the alveolar epithelium are exposed to its highest concentrations and thus, potentially affecting their biophysical properties and structure.

General anesthesia is frequently associated with a transient deterioration of lung mechanics and blood oxygenation [4,5] secondary to atelectasis [6] and ventilation-perfusion mismatch [7]. In this sense, a direct effect of sevoflurane on the alveolar epithelium and pulmonary surfactant has been suggested [8–10] in order to explain those clinical findings.

Sevoflurane, is a fluorinated halogenated anesthetic considered a better option than halothane [11,12], which has been associated with cell damage and lung impairment [13]. Although sevoflurane does not affect the alveolar epithelial structure [14], several reports suggested a pro-inflammatory role leading to different degrees of lung injury [15–17]. Moreover, sevoflurane promoted an early gene up-regulation [18] of endothelin-1, a potent vasoconstrictor recently involved in the inhibition of alveolar fluid reabsorption (AFR) [19].

The aim of our study was to determine whether sevoflurane affects lung surfactant composition and viscosity properties. Particularly, an experimental animal model of short time exposure (30 min) to sevoflurane anesthesia (one minimum alveolar concentration, 1 MAC) is proposed, in order to discriminate the early effect of this anesthetic on alveolar epithelial functionality as well as the biochemical and structural properties of pulmonary surfactant.

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2. Methods

2.1. Animal preparation

Experimental protocol and animal care were approved by the Animal Research Committee of the School of Medicine, Montevideo, Uruguay. Seventy-two male Sprague Dawley rats weighing 310 ± 4.6 g were used in our protocol with free access to food/water and maintained on a 12-h light/12-h dark cycle. Animals were housed in polycarbonate ventilated cages and were handled according to international standards.

2.2. Experimental design

Animals were randomly assigned to one of four groups:

Group 1: Pentobarbital-anesthetized, mechanically-ventilated rats ($n = 18$).

Group 2: Sevoflurane-anesthetized, mechanically-ventilated rats ($n = 18$).

Group 3: Colchicine-treated, pentobarbital-anesthetized, mechanically-ventilated rats ($n = 18$).

Group 4: Pentobarbital-anesthetized, spontaneously breathing rats ($n = 18$).

Once rats were anesthetized with either inhaled sevoflurane (one minimum alveolar concentration, 2–3% sevoflurane) or intraperitoneal pentobarbital (35 mg/kg), Groups 1–3 animals were then supplemented with oxygen (60%) via a T-tube until muscle paralysis was achieved with 0.3 ml intravenous administration of 10 mg ml atracurium. Once muscle paralysis was achieved, animals were mechanically ventilated for 30 min, under the following conditions: tidal volume (VT) of 8 ml/kg, respiratory rate (RR) of 25 breaths/min, and positive end-expiratory pressure of 0 cm H_2O. To evaluate effect of anesthesia on alveolar fluid reabsorption [20] and surfactant secretion [21], pentobarbital-anesthetized rats (Group 3) were dosed with intraperitoneal colchicine (0.25 mg/100 g body weight) 15 h before start of experimental manipulation. Finally, to examine the role of inhaled sevoflurane and mechanical ventilation on lung surfactant lipid profile, Group 4 animals were anesthetized with intraperitoneal pentobarbital (25 mg/kg) and allowed to breathe spontaneously while receiving supplemental oxygen for 30 min via the T-tube.

2.3. Lung mechanics

A tracheal cannula (a polypropylene tube of 50 mm length and 1.5 mm internal diameter) was placed by tracheotomy, inserted 10 mm into the trachea and secured by a lace (2.0 silk suture). Analgesia was achieved by lidocaine hydrochloride (0.3 ml of 2% solution) in the area of incision. Following surgery, pentobarbital or sevoflurane anesthesia was maintained for 30 min. A pneumotachograph with a differential pressure transducer (PNEU01 World Precision Instruments, Inc., Sarasota, FL, USA) was connected to the tracheal cannula in order to measure airflow and lung volume changes. A second differential pressure transducer (PNEU05 World Precision Instruments, Inc., Sarasota, Fl, USA) was used at the side port of the tracheal cannula in order to measure tracheal pressure (Ptr). For the esophageal pressures (Pes) measurements, to separate lung and chest wall compliance, a 30 cm-long water-filled catheter PE-25 was used, with side holes at the tip connected to another differential pressure transducer (Statham P23BC, Hato Rey, Puerto Rico, U.S.A.).

Muscle relaxation was achieved in all ventilated animals with atracurium (0.3 ml of 10 mg/ml solution, i.v.) and mechanical ventilation was performed with 60% oxygen by means of a time-cycled rodent ventilator (SAMAY VR-15, Uruguay) and all data were analyzed using ANADAT data analysis software (RHT Infodat, Montreal, CANADA).

Respiratory mechanics were measured from end-inspiratory occlusions after constant flow inflations as previously described [22]. Briefly, after end inspiratory occlusion, there is an initial fast decrease in tracheal pressure of the respiratory system ($\Delta P1_{RS}$) from the pre-occlusion value down to an inflection point (Pi_{RS}). $\Delta P1_{RS}$ reflects the pressure required to overcome the combination of airway, pulmonary and chest wall resistances. Slow pressure decrease ($\Delta P2_{RS}$) ensues until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the respiratory system (Pel_{RS}). $\Delta P2_{RS}$ reflects the pressure spent on viscoelastic properties of lung and chest wall tissues. Transpulmonary pressure obtained by an esophageal catheter allowed us to differentiate lung (L) and chest wall (W) components from the total respiratory system. Measurements were performed 10 times on each animal and then averaged. Equipment flow resistance (tracheal cannula included) was 0.032 cm H_2O ml^{-1} s. The equipment resistive pressure was subtracted from respiratory system and pulmonary resistive pressures and equals the product of flow resistance by airflow. Equipment dead space was 0.4 ml.

Fig. 1 shows a representative scheme of an end-inspiratory occlusion record.

2.4. Histological analysis

At the end of the MV period, the lungs were extracted and quickly frozen by immersion in liquid nitrogen to perform morphometric analysis, as previous described [23,24]. Briefly, tissues were fixed in Carnoy's solution (ethanol:chloroform:acetic acid, 70:20:10 v/v) at −70 °C for 24 h and then increasing concentrations of ethanol at −20 °C were successively substituted for Carnoy's solution until

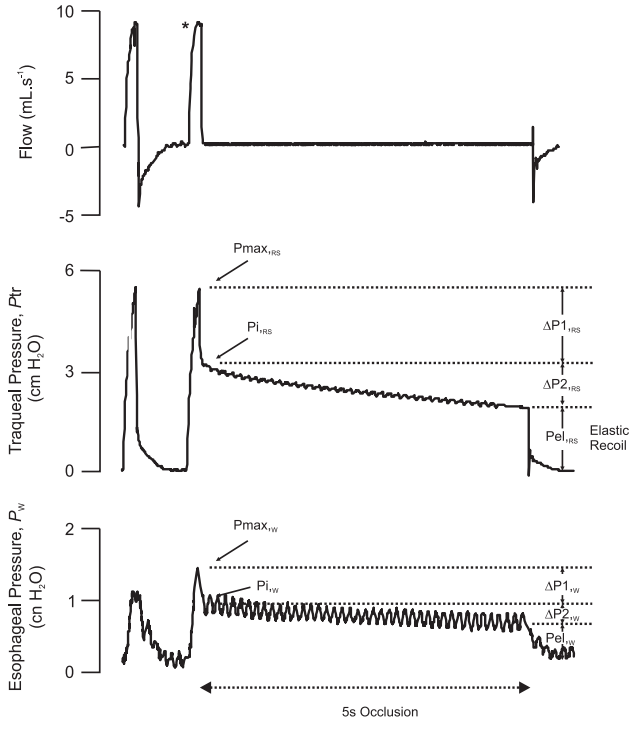

Fig. 1. Lung mechanics. Typical record of end inspiratory occlusion. The figure shows (from top to bottom) flow, tracheal pressure and esophageal pressure. Tracheal pressure record shows, after occlusion, a sudden decrease from peak value ($Pmax_{RS}$) followed by a gradual decrease until plateau pressure was reached (Pel_{RS}). *occlusion, Pmax = Maximal traqueal pressure, Pi_{RS} = tracheal pressure at inflection point, Pel = Elastic recoil pressure, ΔP1 = Viscose pressure decay, ΔP2 = viscoelastic pressure decay. Transpulmonary pressure (Lung) is obtained from the difference between Tracheal and Esophageal pressures.