Pulmonary Pharmacology & Therapeutics xxx (2014) 1–8

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

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

Leonel Malacrida [a], Germán Reta [a], Héctor Piriz [a], Fabiana Rocchiccioli [a], Horacio Botti [b], Ana Denicola [c], Arturo Briva [a,*]

[a] Area de Investigación Respiratoria (AIR), Departamento de Fisiopatología, Hospital de Clínicas, Universidad de la República, Montevideo, Uruguay
[b] Unidad de Cristalografía de Proteínas – Institut Pasteur de Montevideo, Montevideo, Uruguay
[c] Laboratorio de Fisicoquímica Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Article history:
Received 11 July 2013
Received in revised form
6 December 2013
Accepted 24 December 2013

Keywords:
Volatile anesthetics
Lung injury
Pulmonary phospholipids
Mechanical ventilation
Rodents

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.

* Corresponding author. Area de Investigación Respiratoria, Hospital de Clínicas, Departamento de Fisiopatología, Av Italia 2870 piso 15, sala 1, Uruguay. Tel./fax: +39 598 24819161.
E-mail addresses: abriva@hc.edu.uy, arturo_briva@yahoo.com (A. Briva).

1094-5539/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pupt.2013.12.005

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

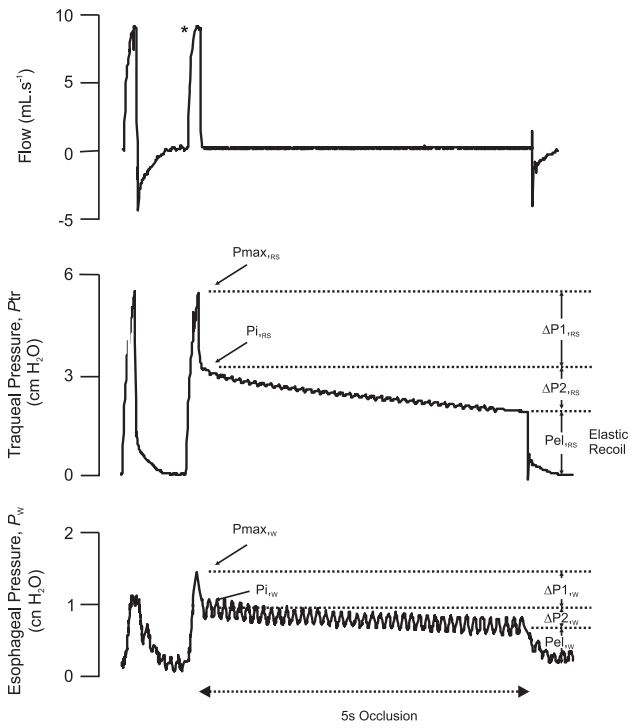

Fig. 1. Lung mechanics. Typical record of end inspiratory occlusion. The figure shows (from top to bottom) flow, tracheal pressure and esophageal pressure. Tracheal pressure record shows, after occlusion, a sudden decrease from peak value ($Pmax_{RS}$) followed by a gradual decrease until plateau pressure 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.

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005

100% ethanol was reached. After fixation, three blocks were performed from midsagittal slices at the level of upper lobe, axial bronchus and lower lobe and they were embedded in paraffin and hematoxylin-eosin stained. One hundred eighty images per study group were obtained by a Nikon Optiphot microscope with a Plan ×40 DIC NA 0.65 objective. Images were processed in Image Pro-Plus software (Media Cybernetics, Inc. Bethesda, MD, USA) and masked for any empty areas, blood vessels and large airways by pseudo-coloring [23]. The same image analysis software was used to threshold images for air spaces and alveolar wall thickness; and expressed in relative units (RU). Areas for air spaces were calculated as a percentage of white/total image area minus masked areas. Alveolar wall thickness was randomly evaluated in those images rejecting measurements associated to full-collapsed alveoli in order to avoid double-sized septae. The histological analysis was performed by an investigator unaware of the sample origin.

2.5. Isolated perfused rat lung model

A second set of animals was distributed among the same subgroups (G1, G2 and G3) as described above. After MV, lungs and heart were removed in block and the isolated lung preparation was performed as previously described [19,23]. Lungs were filled with 5 ml of Bovine Serum Albumin (BSA) solution containing 0.1 mg/ml Evans blue dye (EBD; Sigma). Alveolar fluid reabsorption (AFR) was measured as the differences in concentrations of Evans blue dye-albumin among samples taken from the instilled solution added to the alveolar space.

Mathematical calculations to determine AFR were fully described previously [25].

$$V_0[\mathrm{EBD}]_0 = V_t[\mathrm{EBD}]_t$$

$$V_t = V_0([\mathrm{EBD}]_0/[\mathrm{EBD}]_t)$$

$$J = \frac{V_t - V_0}{t}$$

where, V_0 is the initial known volume instilled into rat air spaces containing a known concentration of Evans blue dye-albumin $[\mathrm{EBD}]_0$. V_t and [EBD]t are the alveolar fluid volume and EBD concentration in the instillate at time t respectively. J is the volume flux during a time period (t).

The perfusate solution was adjusted in normocapnia, 40 mmHg CO_2, bubbled in the perfused liquid, with or without 1 MAC sevoflurane by adding to the perfusate solution the specific amount of sevoflurane (0.3 mM, to avoid any unintended gas dissipation during the perfusate circulation). Fluorescein isothiocyanate (FITC) labeled-albumin was also added to the perfusate to check the capillary barrier integrity along the experiment. Evans blue dye concentration was quantified by its absorbance at 620 nm (Spectrophotometer UV–Vis Shimadzu 160) and FITC concentration by its emission fluorescence at 521 nm (Picofluor fluorometer. Turner Biosystems, CA, USA), in samples obtained at 0 and 60 min from the beginning of the experiment.

Levels of pH, pO_2, and pCO_2 were monitored in the perfusate by a Phox Plus blood gas analyzer (Nova Biomedical; Waltham, MA.)

2.6. Lung lavage procedure

In a third set of animals, after mechanical ventilation and lung mechanics analysis (G1, G2 and G3), the lungs were instilled with one bolus of 5 ml (saline solution, 150 mM NaCl) at room temperature. The lavage was repeated five times with the same initial volume (final recovery ~ 3.5–4.0 ml). The bronchoalveolar lavage fluid (BALF) collected was centrifuged for 10 min at 1000 g and 4 °C to remove cell debris. The supernatant was stored at −20 °C until total protein and phospholipids content was performed.

2.6.1. BALF phospholipids profile

2.6.1.1. Phospholipid (PL) content of BALF was determined by high performance liquid. Chromatography (HPLC – Agilent 1100, Hewlett–Packard, CO, USA, equipped with diode array detector) as previously published with minor modifications [26]. Prior to the analysis, organic extraction of the phospholipids was performed as previously described [27]. The separation was achieved on a silica-based column 250 mm × 4.6 mm I.D. with 5 μm spherical particles and 80 Å pore (Phenomenex, CO). The mobile phase was acetonitrile-methanol-85% phosphoric acid (100:10:1.8, v/v), previously degassed and delivered to the column at a flow-rate of 1.0 ml/min and at 50 °C. The detection wavelength was set at 204 nm and a calibration curve was performed with standard phospholipids samples (Sigma–Aldrich). Total PL content was determined as the algebraic sum of the relative measured components: phosphatidylcholine (PC), lyso-phosphatidylcholine (lyso-PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE).

2.6.2. BALF total protein

Total protein content in BALF samples was estimated by the Bradford method, previously described [28]. BSA solution was used as standard protein; absorbance at 595 nm was measured in samples incubated with Bradford reagent after 10 min of mixing (Finstruments® Multiskan Model 349 96 well Microplate Absorbance Reader.). The protein sample concentration was estimated as the concentration of BSA.

2.7. Thermotropic profile of membranes by diphenylhexatriene anisotropy

Vesicles were made with a selected mix of lipids intended to mimic a pulmonary surfactant sample [29], the mixture were: dipalmitoylphosphatidylcholine (DPPC) : phosphatidylcholine (yolk egg) : cholesterol, 1:1 phospholipid molar ratio and 20% molar of cholesterol with a final concentration of 0.6 mM of total phospholipids. Lipids in methanol-chloroform (1:1 v/v) solution were dried in a CentriVap Concentrator (Labconco. CO), for 1 h at 30 °C. Three independent dispersions of multi lamellar vesicles (MLVs) were prepared by adding Tris buffer 5 mM, pH 7.01 with 150 mM NaCl at 45 °C. At the end, samples were heated at 50 °C and vortexed for 1 h. Finally, the MLVs were extruded through a 1 μm pore membrane eleven times in order to obtain the desired diameter vesicles (Avanti Polars Lipids, Inc). The vesicles were loaded with 1,6-diphenyl-1,3,5-hexatriene (Sigma–Aldrich) in a 1/500 ratio. Empty vesicles (without probe), were prepared for background solution. Anisotropy measurements were determined as the fluorescence emission intensity in parallel (IVV) and perpendicular (IVH) orientations from the exciting beam (vertical orientation), the data was collected 10 times each and then averaged [30]. The probe was excited at 360 nm and the emission was collected at 430 nm.

Anisotropy, r, was calculated as:

$$r = \frac{I_{VV} - G.I_{VH}}{I_{VV} + 2GI_{VH}}$$

and,

$$G = \frac{I_{HV}}{I_{HH}}$$

where G is the monochromator grating correction factor (obtained by setting the excitation polarizer to the horizontal position). The

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005

background was subtracted in each component of the polarized emission (IVV and IVH) before calculation of the anisotropy. The thermotropic behavior between 10 and 50 °C was measured in a Cary Eclipse Fluorimeter (Varian, CO), equipped with four-position cell holder, and with a peltier system for temperature control.

2.8. Data analyses

Data are expressed as means ± SEM; "*n*" represents the number of rats in each group. Data were compared using analysis of variance adjusted for multiple comparisons with the Tukey test. When comparisons were performed between two groups of values, significance was evaluated by the Student's *t*-test.

For anisotropy experiments, we performed analysis of variance (ANOVA) test to compare groups at each temperature level. In addition, a fitting-curve analysis based on Boltzmann's equation (which describes the statistical behavior of a thermodynamic system) was performed to determine if curves are statistically different. In order to identify if the samples were in a normal distribution a Kolmogorov–Smirnov test was performed. A p value ≤ 0.05 was considered significant.

3. Results

3.1. Lung mechanics

To evaluate whether sevoflurane modifies the energy dissipation in the airways compared to an intravenous anesthetic, we determined the lung mechanics of rats ventilated and anesthetized with sevoflurane or pentobarbital sodium as a control group. Compared to control (G1), animals exposed to sevoflurane (G2) showed a decrease in lung compliance with a significant increase in the viscoelastic pressure, static and dynamic elastances in the lung component (Table 1). The chest wall component was unaffected by sevoflurane anesthesia, suggesting that modifications of the respiratory system are a direct consequence of lung tissue modifications. In opposition, lung mechanics of rats pre-treated with colchicine (G3) showed a decrease in respiratory compliance predominantly by a significant impairment of non-tissular components (Table 1).

Table 1
Lung mechanics in ventilated anesthetized animals.

	Ventilatory settings		
	Animals group (n = 13)		
	Group 1	Group 2	Group 3
Flow (mL s^{-1})	10.10 ± 0.04	10.02 ± 0.03	10.04 ± 0.01
Volumen (mL)	2.05 ± 0.03	2.04 ± 0.03	2.04 ± 0.01
Respiratory system (RS)			
$Pmax_{RS}$ (cmH_2O)	11.39 ± 0.63	15.13 ± 0.88**	15.05 ± 1.18*
Pi_{RS} (cmH_2O)	9.12 ± 0.64	12.73 ± 0.94**	11.52 ± 1.00
Pel_{RS} (cmH_2O)	6.93 ± 0.49	9.48 ± 0.80*	10.44 ± 0.87*
$\Delta Ptot_{RS}$ (cmH_2O)	4.60 ± 0.43	5.98 ± 0.33*	4.61 ± 0.35
$\Delta P1_{RS}$ (cmH_2O)	2.41 ± 0.43	2.73 ± 0.28	2.53 ± 0.18
$\Delta P2_{RS}$ (cmH_2O)	2.19 ± 0.16	3.25 ± 0.23**¶¶	2.31 ± 0.17
Est_{RS} (cmH_2O mL^{-1})	3.40 ± 0.21	4.66 ± 0.39*	5.13 ± 0.45*
$Edyn_{RS}$ (cmH_2O mL^{-1})	4.47 ± 0.32	6.26 ± 0.46*	5.85 ± 0.53
ΔE_{RS} (cmH_2O mL^{-1})	1.07 ± 0.08	1.60 ± 0.12**¶¶	1.15 ± 0.09
Lung (L)			
$Pmax_L$ (cmH_2O)	8.76 ± 0.45	12.29 ± 0.89**	10.98 ± 0.30*
Pi_L (cmH_2O)	6.67 ± 0.46	10.06 ± 0.93**	8.90 ± 0.22
Pel_L (cmH_2O)	5.13 ± 0.37	7.38 ± 0.70*	7.23 ± 0.50*
$\Delta Ptot_L$ (cmH_2O)	3.65 ± 0.45	5.10 ± 0.40*	3.75 ± 0.40
$\Delta P1_L$ (cmH_2O)	2.11 ± 0.47	2.41 ± 0.29	2.18 ± 0.15
$\Delta P2_L$ (cmH_2O)	1.54 ± 0.11	2.68 ± 0.31*	1.87 ± 0.30
Est_L (cmH_2O mL^{-1})	2.52 ± 0.19	3.63 ± 0.34*	3.57 ± 0.30
$Edyn_L$ (cmH_2O mL^{-1})	3.27 ± 0.23	4.95 ± 0.47*	4.26 ± 0.30
ΔE_L (cmH_2O mL^{-1})	0.75 ± 0.05	1.32 ± 0.15*¶	0.96 ± 0.04
Chest wall (W)			
$Pmax_W$ (cmH_2O)	2.63 ± 0.32	2.85 ± 0.15	3.97 ± 0.50*
Pi_W (cmH_2O)	2.45 ± 0.30	2.67 ± 0.14	2.71 ± 0.29
Pel_W (cmH_2O)	1.80 ± 0.22	2.10 ± 0.16	3.22 ± 0.86
$\Delta Ptot_W$ (cmH_2O)	0.95 ± 0.15	0.88 ± 0.12	0.85 ± 0.30
$\Delta P1_W$ (cmH_2O)	0.30 ± 0.07	0.31 ± 0.04	0.11 ± 0.02*§
$\Delta P2_W$ (cmH_2O)	0.65 ± 0.11	0.57 ± 0.12	0.63 ± 0.11
Est_W (cmH_2O mL^{-1})	0.88 ± 0.11	1.03 ± 0.07	1.53 ± 0.03**§§
$Edyn_W$ (cmH_2O mL^{-1})	1.20 ± 0.15	1.31 ± 0.06	1.98 ± 0.05**§§
ΔE_W (cmH_2O mL^{-1})	0.32 ± 0.05	0.28 ± 0.06	0.43 ± 0.50

Values represent mean ± SEM.
*$p < 0.05$ Group 2 and 3 compared to Group 1.
**$p < 0.01$ Group 2 and 3 compared to Group 1.
§$p < 0.05$ Group 3 compared to Group 1 and 2.
§§$p < 0.01$ Group 3 compared to Group 1 and 2.
¶$p < 0.05$ Group 2 compared to Group 3.
¶¶$p < 0.05$ Group 2 compared to Group 3.

3.2. Lipid profile and protein content in BALF

Despite other factors, the mechanical properties of lung tissue are directly affected by pulmonary surfactant modifications. Following this reasoning we determined whether the observed alterations in lung mechanics were related to changes in pulmonary surfactant composition.

Phospholipids distribution in BALF was affected by sevoflurane anesthesia. Total content of PLs showed a 1.5 times decrease in G2 group compared to G1 ($p < 0.05$, Table 2).

A significant decrease in PC levels was observed (1.4 and 1.7 times lower, $p < 0.05$) associated with an increase in lyso-PC levels (2.7 and 1.7 times higher, $p < 0.01$) in the G2 group compared to the G4 and G1 groups, respectively.

It was confirmed that mechanical ventilation alone did not alter the total phospholipids content nor the profile of its components (G4 vs. G1) but the changes in BALF phospholipids distribution were due to sevoflurane exposure.

Total protein content although subtly lower in G2 was not significantly different compared with G1 and G4 groups (Table 2).

Based on these results, a direct effect of sevoflurane on pulmonary surfactant metabolism cannot be discarded; therefore, we evaluated the effect of colchicine on pulmonary surfactant as a general inhibitor of cell metabolism.

Animals pre-treated with colchicine showed a significant decrease in total phospholipids content ($p < 0.05$), as well as in PS and PE ($p < 0.01$) with a significant increment in lyso-PC levels ($p < 0.01$) compared to the G1 and G2 groups.

Total phospholipids and proteins in BALF were statistically decreased in G3 compared to G4 groups ($p < 0.05$, Table 2).

Table 2
Phospholipids and Protein values in BAL.

	Animals groups[a]			
	G1	G2	G3	G4
Phospholipids, μg/ml				
PL_{Total}	115.3 ± 9.1	79.0 ± 11.0*	83.9 ± 3.9¶	90.6 ± 14.8
PC	90.1 ± 9.8	51.3 ± 8.8*	66.0 ± 3.8	72.8 ± 12.4
Lyso-PC	4.7 ± 1.9	7.8 ± 0.2**	15.3 ± 1.0Φ	2.9 ± 0.8
PC/Lyso-PC	21.0 ± 4.0	6.6 ± 0.6**Φ	4.3 ± 0.1**Φ	25.6 ± 1.7
PE	13.7 ± 202	10.4 ± 1.9	2.5 ± 0.3**Φ	7.7 ± 1.5
PS	3.0 ± 0.6	2.4 ± 0.3	1.6 ± 0.1**Φ	1.8 ± 0.2
Proteins, mg/ml				
	0.38 ± 0.10	0.22 ± 0.07	0.14 ± 0.03Ø	0.36 ± 0.02

Values represent mean ± SEM
*$p < 0.05$ compared to G1 and G4.
**$p < 0.01$ compared to G1.
Φ$p < 0.01$ compared to G2.
¶$p < 0.05$ compared to G4.
Ø$p < 0.01$ compared to G4.
[a] $n = 8$ per group.

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005

3.3. Lung histology

Gas distribution was achieved through a histological pattern analysis of alveolar collapse and septum thickness. Animals anesthetized with sevoflurane showed an increase in alveolar collapse (gas/tissue ratio) compared to G1 (Fig. 2a and b). Septum thickness was also significantly higher in animals exposed to sevoflurane compared to G1 (Fig. 2a and b).

Animals pre-treated with colchicine showed significant alveolar collapse expressed by gas/tissue ratio and more septum enlargement than the G1 group (Fig. 2a and b). Compared to G2, the G3 group showed less alveolar collapse and similar degrees of septum enlargement (Fig. 2a and b).

3.4. Alveolar fluid reabsorption (AFR)

To evaluate whether sevoflurane could promote alveolar collapse by inhibiting the ability of lungs to clear pulmonary edema, we evaluated the AFR in different groups of rats. AFR was not statistically different in lungs exposed to sevoflurane compared to control rats (Fig. 3a). Alveolar capillary permeability, evaluated by FITC-BSA flux, was not significantly affected by sevoflurane compared to control (Fig. 3b). A significant reduction in AFR was observed in rats pre-treated with colchicine, compared to G1 and G2, without significant differences in FITC fluxes comparing the same groups (Fig. 3a and b).

3.5. Thermotropic profile of membranes by diphenylhexatriene (DPH) anisotropy

To evaluate whether the in vivo effects of sevoflurane could be explained by changes in pulmonary surfactant membrane fluidity, lipid vesicles were made with a lipid mixture imitating pulmonary surfactant, exposed to sevoflurane and analyzed by DPH anisotropy in a thermotropic study. The vesicles in control condition (no sevoflurane exposure) showed a broad ordered-to-disordered transition with an estimated melting point around 33 °C (Fig. 4) (estimated by calculating the second derivative). Sevoflurane (1 and 3 MAC, 0.3 and 0.9 mM) promoted a significant decrease in anisotropy at temperatures below 33 °C, with a significant increase in anisotropy above 40 °C (Fig. 4). As expected, anisotropy changes during the estimated melting transition (Tm) were not identified, however, clear changes in the lateral order phases below (Liquid order, Lo) and above (Liquid disorder, Ld) the Tm were observed.

4. Discussion

Clinical and experimental data have previously demonstrated that sevoflurane anesthesia promotes alveolar collapse and transient hypoxemia [5,10], but the mechanisms underneath are not clearly identified. Based on its biochemical properties, sevoflurane has poor solubility in water [31] and high lipid affinity which promotes its interaction with surfactant and cellular membranes in the alveolar space. In the other hand, pulmonary surfactant and the alveolar epithelium are exposed to the highest anesthetic concentration during general anesthesia. Thus, we exposed rodent lungs to sevoflurane to elucidate whether its effects on lung mechanics are associated to lung edema formation or biochemical/biophysical changes of pulmonary surfactant. Our results show, that changes in pulmonary surfactant composition and structure promote the alveolar collapse observed in ventilated animals anesthetized with sevoflurane while maintaining lung fluid reabsorption.

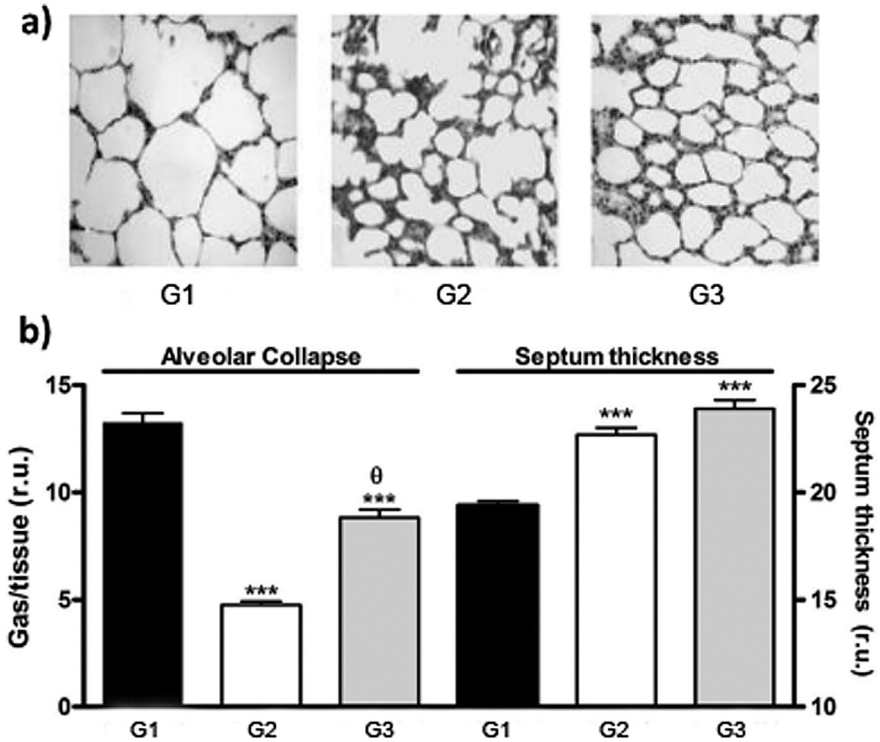

Fig. 2. Histological pattern. (a) Mechanical ventilation with sevoflurane and colchicine administration, both generates lung edema and alveolar distortion compared to control group (representative images). (b) Collapse and septum thickness values were significantly different for sevoflurane and colchicine groups compared to control rats. Bars represent mean ± SEM. G1 = control-pentobarbital-mechanical ventilation group. G2 = sevoflurane-mechanical ventilation group. G3 = colchicine-pentobarbital mechanical ventilation group. $^{***}p < 0.001$ compared to G1. $\theta = p < 0.001$ compared to G2 ($n = 5$).

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005

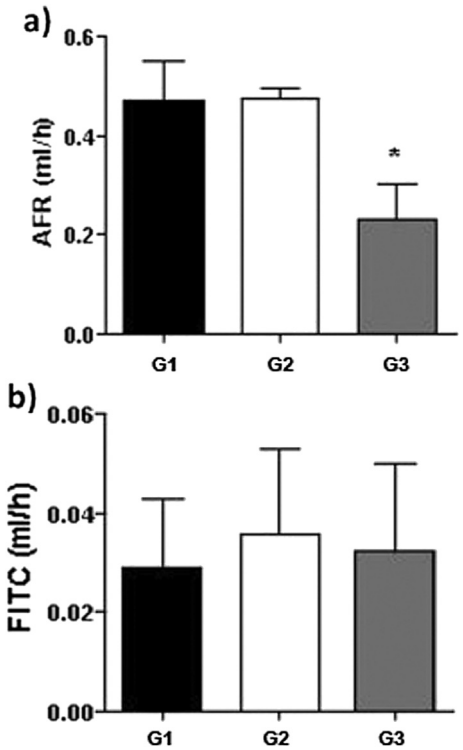

Fig. 3. Alveolar fluid reabsorption (AFR) in isolated lungs. (a) Alveolar fluid reabsorption is not affected by sevoflurane and (b) do not impairs alveolar capillary permeability (right) compared to control group. Colchicine reduces significantly AFR compared to control and sevoflurane. Bars represent mean ± SEM. G1 = control-pentobarbital-mechanical ventilation group. G2 = sevoflurane-mechanical ventilation group. G3 = colchicine-pentobarbital mechanical ventilation group, $^{*}p < 0.05$ ($n = 5$).

Sevoflurane appears as a better volatile anesthetic [14] than halothane and isoflurane, which have been associated with an *in vitro* reduction in surfactant phospholipids synthesis [13,32] and alveolar fluid reabsorption [33]. However, a recent report indicates that sevoflurane increases atelectatic regions and lung inflammation [17], reflecting a potentially induced alveolar instability.

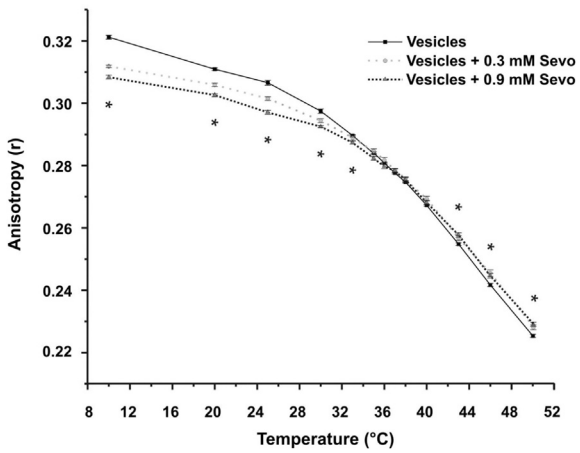

Fig. 4. Thermotropic behavior of vesicles by diphenylhexatriene (DPH) anisotropy. Anisotropy decay of DPH in vesicles during the thermotropic transition study was observed. A significant decrease in the anisotropy is observed by the addition of sevoflurane (1 and 3 MAC, 0.3 and 0.9 mM) at temperatures below 33 °C; and for temperatures above to 40 °C the anisotropy show an increase. However, there are not changes in the anisotropy over the estimated melting transition (Tm) of the membrane. $^{*}p < 0.01$ ($n = 3$).

Based on our results, the animals anesthetized with sevoflurane showed a significant increase on viscoelasticity and lung inhomogeneity expressed by changes in viscoelastic pressure, static and dynamic elastances compared to control and colchicine pretreated rats (Table 1). Since sevoflurane did not modify the pulmonary resistive pressure dissipation, our data reflects changes induced at the peripheral lung tissue and not on airway resistance, as was previously published [10]. Moreover, the observed lung tissue stiffening and the alteration of energy dissipation against the viscoelastic components could be explained by changes in the alveolar environment, as was suggested in a previous report with halothane [8].

Interestingly, after only 30 min of exposure to sevoflurane in a clinical dose, we observed increased levels of lyso-PC associated to a decrease in PC levels (Table 2). This finding is remarkable because increased levels of lyso-PC have been associated with lung injury and abnormal surfactant tensoactive properties secondary to DPPC degradation [34,35].

Indeed, the specific profile of surfactant lipids is fundamental in order to define its ability to lower surface tension [36]. As previously reported, even a modest increase on lyso-PC levels can modify the whole leaflet curvature and inhibit the pulmonary surfactant adsorption capacity by itself [37,38], impairing lung mechanical properties [39].

Considering PC as the most abundant surfactant phospholipid, the accumulation of lyso-PC could reflect an imbalance of surfactant turnover as was suggested also for isoflurane anesthesia [40]. Thus, we decided to evaluate the effect of colchicine, a well-known inhibitor of surfactant phospholipids release by microtubule disruption [41] on a separate group of animals. Colchicine inhibits both the slow surfactant turnover and its rapid release by lung distention [42] and this effect is clearly observed in BALF samples (Table 2).

Animals treated with colchicine showed a typical pattern of cell arrest characterized by low protein secretion and degradation of surfactant lipids, different from that of animals exposed to sevoflurane. However, colchicine promoted alterations of the pulmonary resistive pressure dissipation and less collapse than sevoflurane (Fig. 2a and b) with the highest levels of Lyso-PC in BALF and the lowest PC/Lyso-PC (Table 2) ratio, suggesting that sevoflurane is compromising alveolar stability besides its effect on lipid profile.

In the other hand, halothane promotes the inhibition of active sodium transport across the alveolar epithelium [43] and sevoflurane induces endotelin-1 up-regulation [18] suggesting a potential mechanism of AFR impairment [19]. Thus, alveolar instability induced by lung edema formation secondary to sevoflurane anesthesia could not be excluded.

Interestingly, sevoflurane do not affect AFR or the epithelial integrity in the isolated lung model (Fig. 3), in opposition to the effect of halothane [43]. This is an extremely relevant result because it indicates that the architectural changes observed at the lung tissue by sevoflurane are not related to alveolar edema. Even though colchicine combines the inhibition of surfactant turnover and lung edema reabsorption, G3 rats had less alveolar collapse compared to animals exposed to sevoflurane (Fig. 2b) suggesting a second mechanism besides the increment of lyso-PC.

Volatile anesthetics are lipophilic compounds [44] that can interact with surfactant lipids [45] and promote its dysfunction by a biophysical impairment in a short period of time.

In the selected lipid mixture, sevoflurane promoted a dual change of micro-viscosity: decreased it up to the mean transition point (from 10 to 33 °C) and increased it above the Tm (Fig. 4). In this sense, anisotropy changes should be interpreted as a differential behavior in the pulmonary surfactant structure as a membrane below and above the melting transition point. From 10 to 33 °C the low melting component is disorganized by weakening Van der Waals forces in the

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005

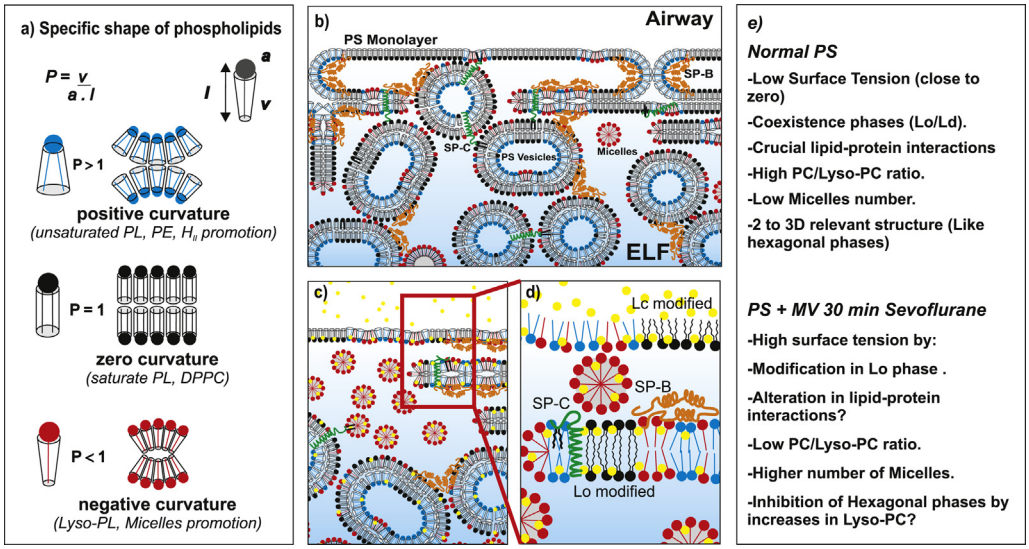

Fig. 5. Proposed scheme of the surfactant dysfunction induced by sevoflurane in the alveolar space (not to scale). (a) Specific shapes of phospholipids (modified from Ref. [53]) determine the development of complex re-arrangements in 2 or 3D of membranes such as bi or monolayers, micelles or inverted hexagonal phases (H_{II}), among others. (b) Normal Surfactant. Schematic view of the pulmonary surfactant at the alveolar air/water interphase. By their lipid–proteins interactions the pulmonary surfactant promotes the final curvature (monolayer at the interphase and multilayers at the hipophase), adsorption (promoting curvature and hexagonal phases) and tensoactive capacity (forming a tight condensed phase). These are related in part to the average shape of the phospholipids mixture (in order to simplify the picture we decided exclude the cholesterol). (c and d) Proposed effect of Sevoflurane and Mechanical Ventilation to the pulmonary surfactant. By the addition of sevoflurane, the lateral order of mono (Liquid condensed phase) and bilayers (Liquid order phases) membranes are altered most likely by weakening lipid–lipid and lipid–protein interactions (associated to changes in the microviscosity), promoting alveolar collapse. Mechanical ventilation aggravates the surfactant dysfunction by an accumulation of lyso-PC, which promotes the appearance of micelles instead of functional structures. Lyso-PC accumulation could inhibit 3D structures affecting the hexagonal phases organization. (e) Summary of properties of native pulmonary surfactant and the effects of 30 min mechanical ventilator with sevoflurane anesthesia. P = specific shape equation, a = area, v = volume and l = length. SP-B = Surfactant Protein B, SP-C = Surfactant Protein C, ELF = Epithelial Lining Fluid, yellow circles = sevoflurane, Lc = Liquid Condensed Phase, Lo = Liquid Order Phase, H_{II} = hexagonal phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

liquid order phase (Lo), which is relevant for surface tension reduction. However, the increased anisotropy from 43 to 50 °C reflects the stabilization of the liquid disorder membrane (Ld) relevant for the inclusion/exclusion cycle of tensoactive material.

The coexistence of Lo/Ld structures *in vitro* are in accordance with our in vivo studies and fairly related with pulmonary surfactant tensoactive properties [46]. Even though a straightforward association between surfactant micro-viscosity and surface tension is not fully established, there is strong evidence supporting that alterations in the lateral structure of the pulmonary surfactant mono/bilayer lead to its dysfunction [47].

Following this reasoning, it is plausible to propose that sevoflurane induces alveolar collapse secondary to a significant surfactant dysfunction by direct biophysical impairment. This effect is magnified by the subsequent mechanical ventilation period that further extends the alveolar collapse by increasing lyso-PC levels [48] (Fig. 5). However, mechanical ventilation and the lack of PEEP in our experimental design is a confounding factor in our study. Is generally accepted that low tidal volume ventilation and zero PEEP promotes alveolar collapse [49], the same evidence suggests that the addition of PEEP did not modify the compliance decay in a rodent model and only periodical lung inflation maneuvers could avoid the unintended alveolar collapse. Since mechanical ventilation itself could affect surfactant turnover, we selected a ventilatory pattern of low tidal ventilation with zero PEEP in order to reduce its secretion [50]. Moreover, the same ventilatory pattern was also protective for AFR [51] and alveolar instability [52] which are present at the onset of lung injury.

In summary, this is the first time where the effects of sevoflurane anesthesia are evaluated in terms of modifications in surfactant lipid profile, lipid–lipid interactions, lung mechanics and histology. Further research should be promoted to fully understand the specific mechanisms of sevoflurane–lipids interactions.

Acknowledgments

The authors acknowledge the collaboration of Javier Hurtado, Anahi Perlas, David Jameson and Jesús Pérez-Gil in the critical reading of the manuscript and valuable suggestions. This work was supported by Comision Sectorial de Investigación Científica, CSIC, Universidad de la Republica (UdelaR) (CSIC-I-2004 and I+D-2006, L.M. and A.B. respectively) and Programa de Desarrollo de las Ciencias Básicas, Uruguay (PEDECIBA, L.M., A.D. and A.B.). L.M. was supported by Comisión Academica de Posgrado (CAP), UdelaR, Uruguay.

The authors have no conflicts of interest.

References

[1] Meyer H. Zur Theorie der Alkoholnarkose. Arch Exp Pathol Pharmakol 1899;42:109–18.
[2] Overton E. Studien über die Narkose Zugleich ein Beitrag zur Allgemeinen Pharmakologie. Jena, Germany: Verlag von Gustav Fisher; 1901.
[3] Cantor RS. Breaking the Meyer-Overton rule: predicted effects of varying stiffness and interfacial activity on the intrinsic potency of anesthetics. Biophys J 2001;80:2284–97.
[4] Hedenstierna G, Edmark L. Mechanisms of atelectasis in the perioperative period. Best Pract Res Clin Anaesthesiol 2010;24:157–69.
[5] Hedenstierna G. Effects of anaesthesia on respiratory function. Ba Clin Anaesth 1996;10:1–16.
[6] Gunnarsson L, Tokics L, Gustavsson H, Hedenstierna G. Influence of age on atelectasis formation and gas exchange impairment during general anaesthesia. Br J Anaesth 1991;66:423–32.
[7] Rothen HU, Sporre B, Engberg G, Wegenius G, Hedenstierna G. Airway closure, atelectasis and gas exchange during general anaesthesia. Br J Anaesth 1998;81:681–6.
[8] Reta GS, Riva JA, Piriz H, Medeiros AS, Rocco PR, Zin WA. Effects of halothane on respiratory mechanics and lung histopathology in normal rats. Br J Anaesth 2000;84:372–7.
[9] Tobin WR, Kaiser HE, Groeger AM, Lema MJ. The effects of volatile anesthetic agents on pulmonary surfactant function. In Vivo 2000;14:157–63.

[10] Correa FC, Ciminelli PB, Falcao H, Alcantara BJ, Contador RS, Medeiros AS, et al. Respiratory mechanics and lung histology in normal rats anesthetized with sevoflurane. J Appl Physiol 2001;91:803–10.
[11] Girgis Y, Frerk CM, Pigott D. Redistribution of halothane and sevoflurane under simulated conditions of acute airway obstruction. Anaesthesia 2001;56:613–5.
[12] Valtcheva R, Stephanova E, Jordanova A, Pankov R, Altankov G, Lalchev Z. Effect of halothane on lung carcinoma cells A 549. Chem Biol Interact 2003;146:191–200.
[13] Molliex S, Crestani B, Dureuil B, Bastin J, Rolland C, Aubier M, et al. Effects of halothane on surfactant biosynthesis by rat alveolar type II cells in primary culture. Anesthesiology 1994;81:668–76.
[14] Takala RS, Soukka HR, Kirvela OA, Kujari HP, Pelliniemi LJ, Kaapa PO, et al. Alveolar integrity and ultrastructure in pigs remain undamaged after exposure to sevoflurane. Acta Anesthesiol Scand 2002;46:1137–43.
[15] Kotani N, Takahashi S, Sessler DI, Hashiba E, Kubota T, Hashimoto H, et al. Volatile anesthetics augment expression of proinflammatory cytokines in rat alveolar macrophages during mechanical ventilation. Anesthesiology 1999;1:187–97.
[16] Takala RS, Soukka HR, Salo MS, Kirvela OA, Kaapa PO, Rajamaki AA, et al. Pulmonary inflammatory mediators after sevoflurane and thiopentone anaesthesia in pigs. Acta Anesthesiol Scand 2004;48:40–5.
[17] Kalimeris K, Christodoulaki K, Karakitsos P, Batistatou A, Lekka M, Bai M, et al. Influence of propofol and volatile anaesthetics on the inflammatory response in the ventilated lung. Acta Anaesthe Scand 2011;55:740–8.
[18] Sakamoto A, Jun-ichi I, Nishikawa A, Reiko H, Emi I, Yanagisawa Y, et al. Influence of inhalation anesthesia assessed by comprehensive gene expression profiling. Gene 2005;356:39–48.
[19] Comellas AP, Briva A, Dada LA, Butti ML, Trejo HE, Yshii C, et al. Endothelin-1 impairs alveolar epithelial function via endothelial ETB receptor. Am J Respir Crit Care Med 2009;179:113–22.
[20] Saldias FJ, Lecuona E, Comellas AP, Ridge KM, Sznajder JI. Dopamine restores lung ability to clear edema in rats exposed to hyperoxia. Am J Respir Crit Care Med 1999;159:626–33.
[21] Andreeva AV, Kutuzov MA, Voyno-Yasenetskaya TA. Regulation of surfactant secretion in alveolar type II cells. Am J Physiol Lung Cell Mol Physiol 2007;293:L259–71.
[22] Bates JHT, Rossi A, Milic-Emili J. Analysis of the behavior of the respiratory system with constant inspiratory flow. J Appl Physiol 1985;58:1840–8.
[23] Briva A, Santos C, Malacrida L, Rocchiccioli F, Soto J, Angulo M, et al. Adenosine triphosphate-dependent calcium signaling during ventilator-induced lung injury is amplified by hypercapnia. Exp Lung Res 2011;37:471–8.
[24] Burburan SM, Xisto DG, Ferreira HC, Riva Ddos R, Carvalho GM, Zin WA, et al. Lung mechanics and histology during sevoflurane anesthesia in a model of chronic allergic asthma. Anesth Anal 2007;104:631–7.
[25] Rutschman D, Olivera W, Sznajder JI. Active transport and passive liquid movement in isolated perfused rat lungs. J Appl Physiol 1993;75:1574–80.
[26] Shafiq URR. Rapid isocratic method for the separation and quantification of major phospholipid classes by high-performance liquid chromatography. J Chromatogr 1991;567:29–37.
[27] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
[28] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem ;72:248–54.
[29] Malacrida L, Botti H, Rochiccioli F, Denicola A, Briva A. Halogenated anesthetics impairs biophysical properties of a membrane model of pulmonary surfactant. Biophys J 2011;100:505a–6a.
[30] Shinitzky M, Dianoux AC, Gitler C, Weber G. Microviscosity and order in the hydrocarbon region of micelles and membranes determined with fluorescent probes. I. Synthetic micelles. Biochemistry 1971;25:2106–13.
[31] Wüstneck N, Wüstneck R, Pison U, Möhwald. On the dissolution of vapors and gases. Langmuir 2007;23:1815–23.
[32] Molliex S, Crestani B, Dureuil B, Rolland C, Aubier M, Desmonts JM. Differential effects of isoflurane and i.v. anaesthetic agents on metabolism of alveolar type II cells. Br J Anaesth 1999;82:767–9.
[33] Rezaiguia-Delclaux S, Jayr C, Luo DF, Saidi NE, Meignan M, Duvaldestin P. Halothane and isoflurane decrease alveolar epithelial fluid clearance in rats. Anesthesiology 1998;88:751–60.
[34] Gregory TJ, Longmore WJ, Moxley MA, Whitsett JA, Reed CR, Fowler III AA, et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991;88:1976–81.
[35] Arbibe L, Koumanov K, Vial D, Rougeot C, Faure G, Havet N, et al. Generation of lysophospholipids from surfactant in acute lung injury is mediated by type-II phospholipase A2 and inhibited by a direct surfactant protein A-phospholipase A2 protein interaction. J Clin Invest 1998;6:1152–60.
[36] Perez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. Biochim Biophys Acta 2008;1778:1676–95.
[37] Gruner SM. Stability of lyotropic phases with curved interfaces. J Phys Chem 1989;93:7562–70.
[38] Biswas SC, Rananavare SB, Hall SB. Differential effects of lysophosphatidylcholine on the adsorption of phospholipids to an air/water interface. Biophys J 2007;92:493–501.
[39] Fornasier M, Puccini P, Razzetti R, Acerbi D, Bongrani S, Ventura P. Evaluation of potentially injurious effects of exogenous surfactant lysophospholipids on the alveolar epithelium and pulmonary mechanics. Bio Neonate 1997;71:337–44.
[40] Yang T, Li Y, Liu Q, Tao J, Wu W, Huang H. Isoflurane aggravates the decrease of phosphatidylcholine synthesis in alveolar type II cells induced by hydrogenperoxide. Drug Metabol Drug Interact 2001;18:243–9.
[41] Brown LA, Pasquale SM, Longmore WJ. Role of microtubules in surfactant secretion. J Appl Physiol 1985;58(6):1866–73.
[42] Corbet AJ, Voelker RM, Murphy FM, Owens ML. Inhibition by colchicine of phospholipid secretion induced by lung distension. J Appl Physiol 1988;65(4):1710–5.
[43] Molliex S, Dureuil B, Aubier M, Friedlander G, Desmonts JM, Clerici C. Halothane decreases Na,K-ATPase, and Na channel activity in alveolar type II cells. Anesthesiology 1998;88:1606–13.
[44] Suezaki Y, Shibata A, Kamaya H, Ueda I. Atypical Langmuir adsorption of inhalation anesthetics on phospholipid monolayer at various compressional states: difference between alkane-type and ether-type anesthetics. Biochim Biophys Acta 1985;817:139–46.
[45] Stephanova E, Valtcheva-Sarker R, Topouzova-Hristova T, Lalchev Z. Influence of volatile anaesthetics on lung cells and lung surfactant. Biotech Biotechnol Equip 2007;21:393–8.
[46] Bernardino de la Serna J, Perez-Gil J, Simonsen AC, Bagatolli LA. Cholesterol rules: direct observation of the coexistence of two fluid phases in native pulmonary surfactant membranes at physiological temperatures. J Biol Chem 2004;279:40715–22.
[47] Casals C, Cañadas O. Role of lipid ordered/disordered phase coexistence in pulmonary surfactant function. Biochim Biophys Acta 2012;1818:2550–62.
[48] Naz S, Garcia A, Erazo M, Ruiz-Cabello J, Esteban A, Lorente JA, et al. A novel non-targeted metabolomic approach in ventilator induced lung injury: mass spectrometric characterization of fatty amide, sphinganine and lysophosphatidylcholine. Am J Respir Crit Care Med 2012;185:A6724.
[49] Reiss LK, Kowallik A, Uhlig S. Recurrent recruitment manoeuvres improve lung mechanics and minimize lung injury during mechanical ventilation of healthy mice. PLoS ONE 2011;6:e24527.
[50] Savov J, Silbajoris R, Young SL. Mechanical ventilation of rat lung: effect on surfactant forms. Am J Physiol 1999;277:L320–6.
[51] Lecuona E, Saldías F, Comellas A, Ridge K, Guerrero C, Sznajder JI. Ventilator-associated lung injury decreases lung ability to clear edema in rats. Am J Respir Crit Care Med 1999;159:603–9.
[52] Allen GB, Pavone LA, DiRocco JD, Bates JH, Nieman GF. Pulmonary impedance and alveolar instability during injurious ventilation in rats. J Appl Physiol 2005;99(2):723–30.
[53] Mouritsen OG. Lipids, curvature, and nano-medicine. Eur J Lipid Sci Technol 2011;113(10):1174–87.

Please cite this article in press as: Malacrida L, et al., Sevoflurane anesthesia deteriorates pulmonary surfactant promoting alveolar collapse in male Sprague–Dawley rats, Pulmonary Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pupt.2013.12.005