Lung Mechanics and Histology During Sevoflurane Anesthesia in a Model of Chronic Allergic Asthma

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BACKGROUND: There are no studies examining the effects of sevoflurane on a chronically inflamed and remodeled airway, such as that found in asthma. In the present study, we sought to define the respiratory effects of sevoflurane in a model of chronic allergic asthma. For this purpose, pulmonary mechanics were studied and lung morphometry analyzed to determine whether the physiological modifications reflected underlying morphological changes.

METHODS: Thirty-six BALB/c mice (20–25 g) were randomly divided into four groups. In OVA groups, mice were sensitized with ovalbumin and exposed to repeated ovalbumin challenges. In SAL groups, mice received saline using the same protocol. Twenty-four hours after the last challenge, the animals were anesthetized with pentobarbital sodium (PENTO, 20 mg/kg i.p.) or sevoflurane (SEVO, 1 MAC). Lung static elastance (E_{st}), resistive (ΔP_1) and viscoelastic/inhomogeneous (ΔP_2) pressure decreases were analyzed by an end-inflation occlusion method. Lungs were fixed and stained for histological analysis.

RESULTS: Animals in the OVASEVO group showed lower ΔP_1 (38%), ΔP_2 (24%), and E_{st} (22%) than animals in the OVAPENTO group. Histology demonstrated greater airway dilation (16%) and a lower degree of alveolar collapse (25%) in the OVASEVO compared with OVAPENTO group. ΔP_1 was lower (35%) and airway diameters larger (12%) in the SALSEVO compared with SALPENTO group.

CONCLUSION: Sevoflurane anesthesia acted both at airway level and lung periphery reducing (ΔP_1 and ΔP_2 pressures, and E_{st} in chronic allergic asthma.

(Anesth Analg 2007;104:631–7)

Sevoflurane is a potent inhaled anesthetic that has been widely used in adults and especially in children, because it provides faster induction and awakening (1), and causes less airway irritation than other inhaled anesthetics (2,3).

Asthma is a major public health issue with high and increasing prevalence rates (4) and a concomitant increase in morbidity and mortality (5). Studies have shown the prevalence of asthma among adults is 11%, 6 being even higher among children (7). These data, combined with the well-documented poor adherence to treatment (8), suggest that anesthesiologists will see more adverse events due to asthma in clinical daily practice.

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Accepted for publication November 9, 2006.

Supported by the Centers of Excellence Program (PRONEX-FAPERJ), Brazilian Council for Scientific and Technological Development (CNPq), Carlos Chagas Filho Rio de Janeiro State Research Supporting Foundation (FAPERJ).

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DOI: 10.1213/01.ane.0000255073.96001.cb

Allergic asthma is the type experienced by approximately 80% of asthmatics presenting eosinophilic inflammation and structural changes of the airway wall (9). Inflammation in chronic asthma involves the activation of airway cells, including T-cells, eosinophils, mast cells, macrophages, epithelial cells, fibroblasts, and even bronchial smooth muscle cells. Chronic inflammation is associated with injury and repair of the bronchial epithelium, which results in structural and functional changes known as remodeling. These structural changes include smooth muscle hypertrophy, mucous gland hyperplasia, blood vessel proliferation, and sub-basement membrane collagen deposition (10). The underlying chronic inflammatory process compromises not only central airways but also distal airways and lung parenchyma (11,12).

Studies relating sevoflurane administration and bronchial hyperresponsiveness have produced conflicting results (13,14), and there are no reports describing its effects on chronically inflamed and remodeled airways as evidenced in asthma.

The aim of this study was to determine the respiratory effects of sevoflurane in a murine model of chronic allergic asthma. For this purpose, we performed a randomized controlled study in which global variables, such as total resistive pressure as well as relative contributions of airways and tissues to modify the lungs' mechanical profile, were evaluated. Additionally, we studied lung histology and correlated morphological

changes with physiological variables to define the sites of action of sevoflurane.

METHODS

Animal Preparation

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Center, Federal University of Rio de Janeiro. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences, USA.

Thirty-six BALB/c mice weighing 15–20 g, 6 wk of age were randomly divided into two groups. In the ovalbumin (OVA) group, mice were sensitized, using an adjuvant-free protocol, by the intraperitoneal injection of ovalbumin (10 μg in 0.1 mL saline) on each of 7 alternate days (11,15). Forty days after the beginning of sensitization, 20 μg ovalbumin in 20 μL sterile saline was intratracheally instilled (11). This procedure was performed three times with a 3-day interval between instillations. The control group (SAL) received saline using the same protocol. The animals were housed for 47 days in microisolator cages (filter tops) that inhibited spread of infectious agents from one cage to another, as well as from investigators to animals and *vice versa*. All animals received uniform handling, identical feed, water and a 12-h light–dark cycle. Furthermore, to avoid lung infection, all substances and surgical material were sterilized.

Twenty-four hours after the last challenge, nine mice in the OVA group (OVASEVO group) and nine in the SAL group (SALSEVO group) were anesthetized with sevoflurane 1 minimum alveolar concentration (MAC) administered by mask using a flow of air passed through a calibrated sevoflurane vaporizer (HB, RJ, Brazil). When an appropriate level of anesthesia was achieved, tracheotomy was established and a snugly fitting cannula (0.8 mm ID) was introduced into the trachea. A T-piece system, which did not cause any appreciable change in tracheal pressure, was used to deliver sevoflurane to the animal.

Similarly, nine animals in the OVA group (OVAPENTO group) and nine in the SAL group (SALPENTO group) were sedated with diazepam (1 mg i.p.), anesthetized with pentobarbital sodium (20 mg/kg i.p.), and tracheotomized as described above.

In the first minutes of the experiments, with the animal breathing spontaneously, the level of the anesthesia was assessed by evaluating its response to light, the position of the nictitating membrane, and by movement response to stimulation of the middle third of the tail with a tail clamp.

Muscle relaxation was achieved with vecuronium bromide (5 μg/kg IV), and a constant flow ventilator (Samay 15, Universidad de la Republica, Montevideo, Uruguay) provided mechanical ventilation with a frequency of 100 breaths/min. Special care was taken to keep tidal volume (V_T = 0.2 mL) and airflow (V' = 1 mL/s) constant to avoid the effects of different flows, volumes, and inspiratory duration on the measured variables.

After muscle relaxation, adequate depth of anesthesia was assessed by evaluating pupil size and light reactivity. Mice rested in the supine position on a surgical table. A positive end-expiratory pressure of 2 cm H_2O was applied and the anterior chest wall surgically removed.

A pneumotachograph (1.5 mm ID, length = 4.2 cm, distance between side ports = 2.1 cm) was connected to the tracheal cannula for the measurements of V' and V_t. The pressure gradient across the pneumotachograph was determined by a differential pressure transducer (SCIREQ, SC-24, Montreal, Canada). V_t was obtained by integration of the V' signal. The flow resistance of the equipment (R_{eq}), tracheal cannula included, was constant up to flow rates of 26 mL/s, and amounted to 0.12 cm H_2O/mL/s. Equipment resistive pressure (R_{eq}/V') was subtracted from pulmonary resistive pressure so that the results represented intrinsic values. Tracheal pressure (P_{tr}) was measured with the differential pressure transducer SCIREQ (SC-24). The equipment dead-space was 0.1 mL. All signals were filtered (100 Hz) and amplified in a four-channel conditioner (SC-24, SCIREQ). Flow and pressure signals were then sampled at 200 Hz with a 12-bit analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA), and stored on a microcomputer. All data were collected using LABDAT software (RHT-InfoData, Montreal, Quebec, Canada).

Measurement of Pulmonary Mechanics

Pulmonary mechanics were measured by the end-inflation occlusion method (16). In an open chest preparation, P_{tr} reflects transpulmonary pressure (P). Briefly, after end-inspiratory occlusion, there is an initial fast decrease in P (ΔP_1) from the preocclusion value down to an inflection point (P_i), followed by a slow pressure decay (ΔP_2), until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lungs (P_{el}). ΔP_1 reflects the pressure used to overcome the airway resistance, which is computed by dividing ΔP_1 by flow. ΔP_2 reproduces the pressure spent by stress relaxation, or viscoelastic properties of the lung, together with a small contribution of pendelluft (16). Total pressure decrease (ΔP_{tot}) is equal to the sum of ΔP_1 and ΔP_2. Lung static elastance (E_{st}) was calculated by dividing P_{el} by V_t. Pulmonary mechanics measurements were performed 10 times in each animal. Values are presented as means ± SEM for each group. All data were analyzed using ANADAT data analysis software (RHT-InfoData). The experiments did not last more than 30 min.

Lung Histology and Morphometry

Immediately after the determination of respiratory mechanics, heparin (1000 IU) was injected IV. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. Then, the lungs were removed *en bloc* at end-expiration. Two investigators, who were unaware of the origin of the material, examined the samples microscopically.

The lungs were quick-frozen by immersion in liquid nitrogen, fixed with Carnoy's solution and embedded in paraffin. Four-micrometer thick tissue slices were obtained by means of a microtome and stained with hematoxylin-eosin. Morphometric analysis was performed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines (known length) coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The volume fractions of collapsed and normal pulmonary areas were determined by the point-counting technique (17) done at a magnification of 200× across 10 random, noncoincident microscopic fields. The number of points that were in normal or collapsed alveoli was counted and divided by the total number of points in the grid.

Four intraparenchymatous airways from each animal were viewed at a magnification of 400×. Points on airway lumen were counted (NP), as well as the number of intercepts (NI) of the lines with epithelial basal membrane. Because the NI of the lines with the epithelial basal membrane is proportional to the airway perimeter, and the NP on airway lumen is proportional to airway area, the magnitude of bronchoconstriction (contraction index, CI) was computed by the relationship $CI = NI/\sqrt{NP}$ (18,19).

Statistical Analysis

SigmaStat 3.0 statistical software package (Jandel Corporation, San Raphael, CA) was used. The normality of the data (Kolmogorov-Smirnov test with Lilliefors' correction) and the homogeneity of variances (Levene median test) were tested. Both conditions were satisfied and a two-way analysis of variance (ANOVA) was used. If multiple comparisons were required, Tukey test was applied. Correlation between mechanical and histological data was determined by Spearman correlation test. A P value <0.05 was considered significant.

RESULTS

During the experiments, the animals did not lose weight (initial weight: 15–20 g, final weight: 20–25 g). There was no statistically significant difference in flow (SALPENTO = 1.01 ± 0.01 mL/s, OVAPENTO = 1.01 ± 0.01 mL/s; SALSEVO = 1.01 ± 0.01 mL/s and OVASEVO = 1.00 ± 0.01 mL/s) and volume (SALPENTO = 0.20 ± 0.01 mL, OVAPENTO = 0.20 ± 0.01 mL, SALSEVO = 0.20 ± 0.01 mL and OVASEVO = 0.20 ± 0.01 mL) among all groups. E_{st}, ΔP_1, ΔP_2, and ΔP_{tot} were higher in OVA than in SAL groups. Animals anesthetized with sevoflurane had lower values of E_{st}, ΔP_1, ΔP_2, and ΔP_{tot} than those anesthetized with pentobarbital sodium. Moreover, ΔP_1 was lower in the SALSEVO than in the SALPENTO group, while E_{st}, ΔP_2, and ΔP_{tot} were similar in these groups (Fig. 1).

The fraction of area of alveolar collapse was higher in the OVA than in the SAL groups (Table 1). OVA groups showed smaller central and distal airway diameters than those found in the SAL groups. The OVASEVO group had wider central and distal airways and a smaller amount of alveolar collapse associated with a higher degree of normal areas than those found in the OVAPENTO group (Table 1 and Fig. 2). Furthermore, SALSEVO animals showed larger central airway diameters than those in the SALPENTO group (Table 1).

Considering all groups together, ΔP_1, ΔP_2, and E_{st} were well correlated with the fractional area of alveolar collapse. Additionally, ΔP_1 was correlated with the contraction index (Fig. 3).

DISCUSSION

This study provides evidence that sevoflurane anesthesia decreases the pressure necessary to overcome airway resistance, viscoelasticity and/or inhomogeneity and static elastance in a murine model of chronic allergic asthma. These functional findings were supported by histological analysis.

In vivo models offer valuable information in asthma pathophysiology and treatment, because they allow the study of the dynamics of certain phenomena and the relation of structure to function (20). Nevertheless, these studies are not always performed in asthmatic humans because of the difficulties in assessing the airway wall noninvasively. Hence, animal models have been used to mimic specific traits of the human disease (21).

In our study, we developed a chronic allergic asthma model in which mice were chronically sensitized and repeatedly challenged with ovalbumin instilled intratracheally, instead of intranasally, to certify that each animal received the same amount of allergen. This model was previously described (11) and reproduces many characteristics of the human disease, such as epithelial denudation, infiltration and activation of eosinophils, macrophages, and T lymphocytes in the airway mucosa and lumen, along with hypertrophy and hyperplasia of airway smooth muscle, goblet cells, and submucosal glands (10,11). In this model of chronic allergic asthma, resistive pressure increased by bronchoconstriction (Table 1 and Fig. 1) as well as by airway remodeling (9,11). Although this is a chronic preparation, in accordance with the most commonly used protocols (11,15), we should be conscious that the morphological and functional changes observed can vary with even longer preparations and with diverse mouse strains (11,20,22).

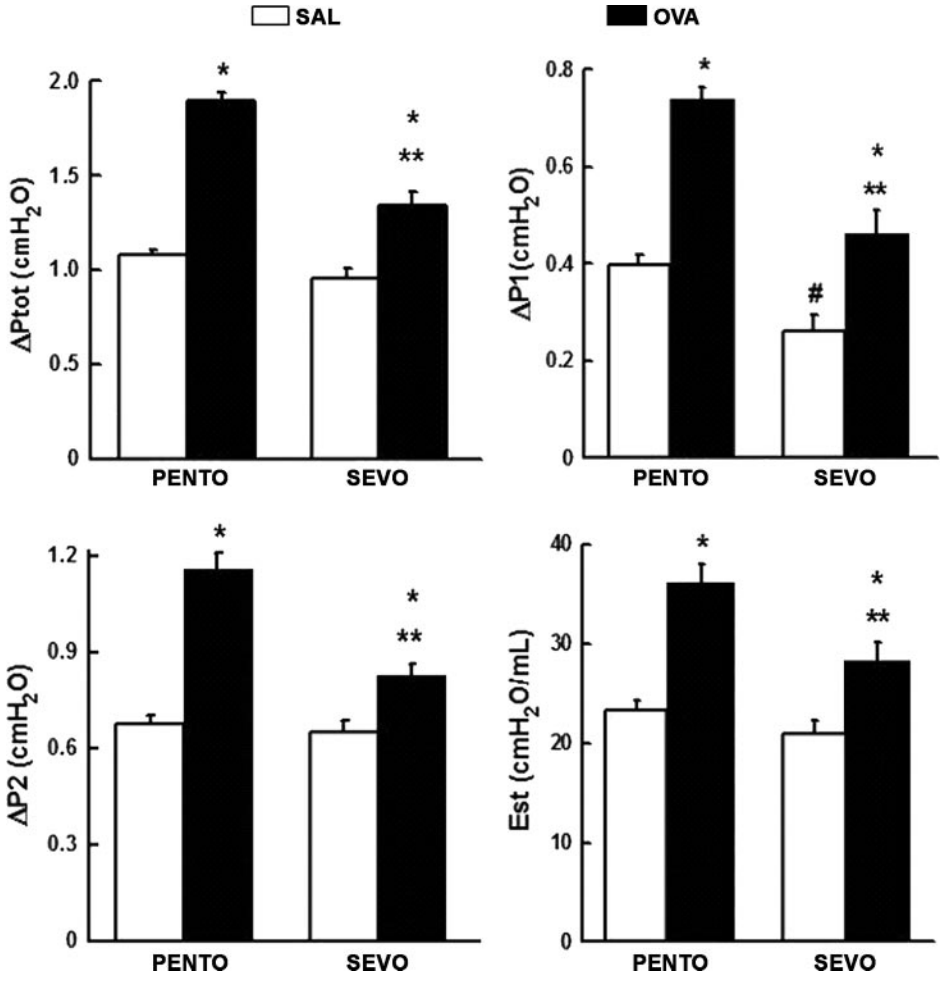

Figure 1. Bars are means + SEM of 9 animals in each group (10 determinations per animal). ΔP_{tot}, ΔP_1, and ΔP_2 = total, resistive, and viscoelastic/inhomogeneous pressures, respectively. E_{st} = static elastance. In the OVA group mice were sensitized and repeatedly challenged with intratracheal instillation of ovalbumin. The SAL group received saline using the same protocol. Animals were anesthetized with pentobarbital sodium (PENTO) or sevoflurane (SEVO) 1 MAC. *$P < 0.05$ when compared with correspondent SAL group. **$P < 0.001$ when compared with OVAPENTO group. #$P < 0.005$ when compared with SALPENTO group.

Table 1. Morphometric Parameters

Group	Normal area (%)	Alveolar collapse (%)	Contraction index
SALPENTO	92.1 ± 0.87	7.8 ± 0.87	1.8 ± 0.03
SALSEVO	95.1 ± 0.63	4.8 ± 0.63	1.6 ± 0.04§
OVAPENTO	73.4 ± 1.45†	26.2 ± 1.47†	2.4 ± 0.07†
OVASEVO	79.8 ± 1.67*‡	19.7 ± 1.53*‡	2.1 ± 0.04*‡

Values are means ± SEM of 9 animals in each group. Data were gathered from 10 random, noncoincident fields per mouse. In the OVA groups, mice were sensitized with ovalbumin and exposed to repeated challenges with intratracheal instillation of ovalbumin. The SAL groups received saline using the same protocol. Mice were anesthetized with pentobarbital sodium or sevoflurane.

SALPENTO = saline/pentobarbital; SALSEVO = saline/sevoflurane; OVAPENTO = ovalbumin/pentobarbital; OVASEVO = ovalbumin/sevoflurane.

* $P < 0.001$ when compared with SALSEVO group.

† $P < 0.001$ when compared with SALPENTO group.

‡ $P < 0.001$ when compared with OVAPENTO group.

§ $P < 0.005$ compared with SALPENTO group.

Pentobarbital sodium has been considered an ideal control drug, because it has no effect on airway baseline tone (23) and causes no modification in respiratory mechanics or lung morphometry (24). Vecuronium was chosen because it seems to bind to M_3 receptors more, or at least to the same extent, as M_2 receptors , and thus, it does not induce bronchospasm (25).

Respiratory mechanical variables were measured by the end-inflation occlusion method and $PAco_2$ ranged between 43 and 45 mm Hg in all animals. Consequently, the mechanical changes could not be attributed to either hyper- or hypocapnia.

In the literature, apart from three cases reporting a beneficial action of sevoflurane in refractory status

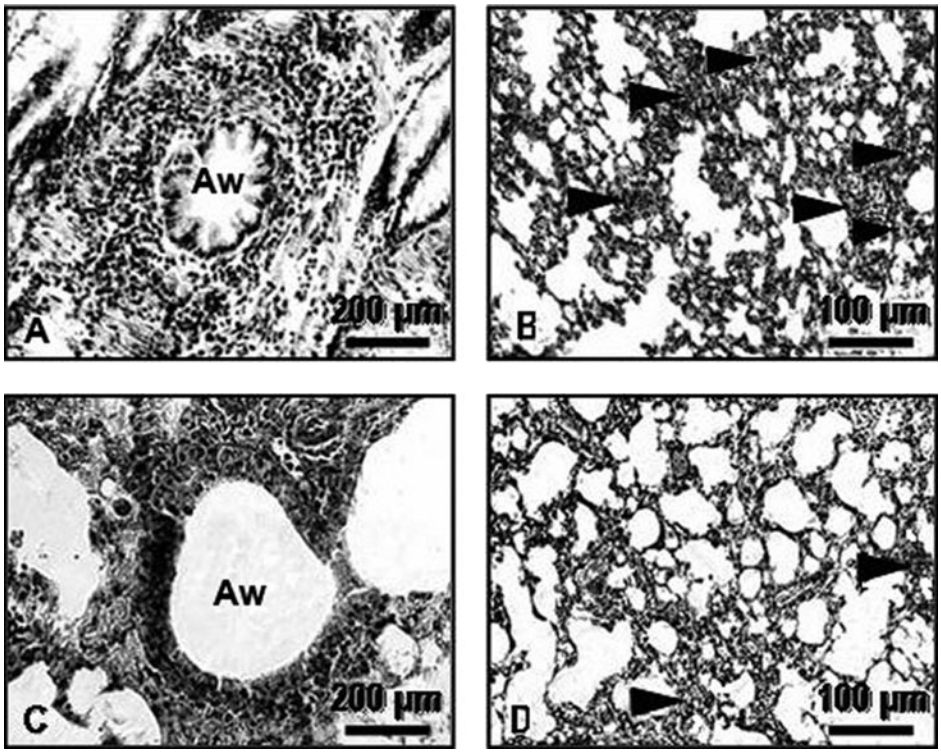

Figure 2. Representative photomicrographs of airways (A and C), and distal lung parenchyma (B and D) from mice sensitized and exposed to repeated challenges with intratracheal instillation of ovalbumin (OVA). In A and B, animals were anesthetized with pentobarbital sodium (OVAPENTO, $n = 9$) and in C and D with sevoflurane (OVASEVO, $n = 9$) 1 MAC. The airway (Aw) was constricted and the amount of alveolar collapse (arrowheads) was higher in OVAPENTO than in OVASEVO group. Mice showed a peribronchial accumulation of inflammatory cells, including eosinophils and mononuclear cells. Hematoxylin-eosin staining. Original magnification: A and C = ×400; B and D = ×200. Bars: A and C = 200 µm; B and D = 100 µm.

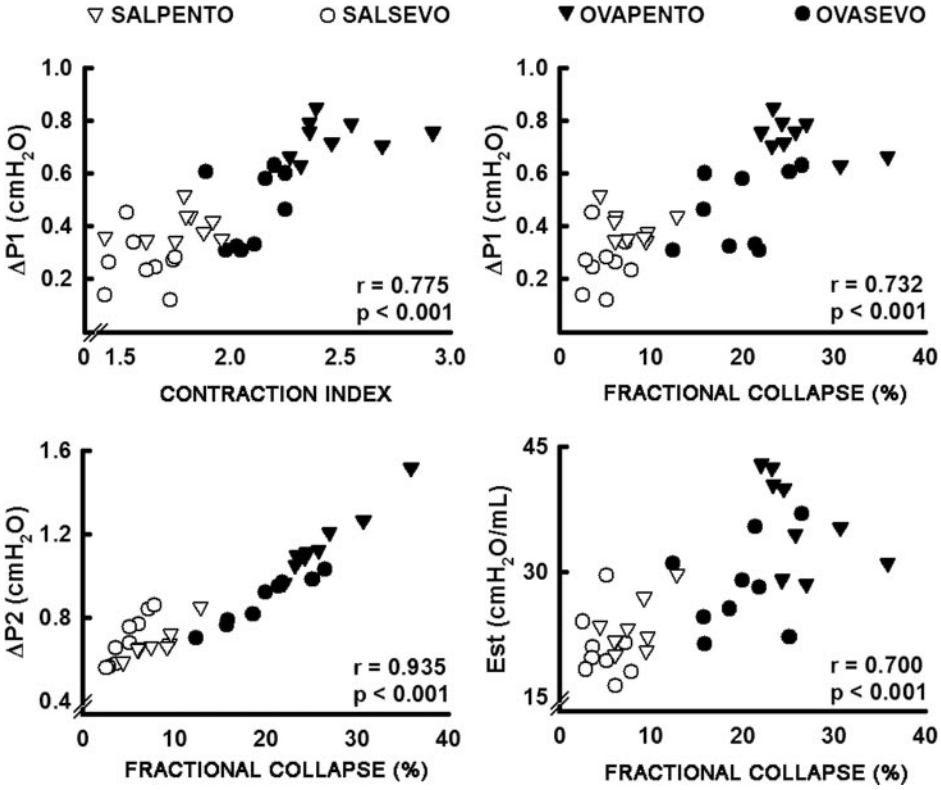

Figure 3. Correlations between mechanical and morphological data obtained through Spearman's correlation test in SALPENTO (saline/pentobarbital), SALSEVO (saline/sevoflurane), OVAPENTO (ovalbumin/pentobarbital) and OVASEVO (ovalbumin/sevoflurane) groups. Pulmonary static elastance (E_{st}), resistive (ΔP_1) and viscoelastic/inhomogeneous (ΔP_2) pressures values were correlated with the fraction of area of alveolar collapse and contraction index.

asthmaticus (26–28), wherein airway remodeling was assumed to be present, studies have analyzed the effects of sevoflurane on acute models of bronchial hyperreactivity, i.e., in the absence of remodeled airways, that did not reproduce chronic asthma features. Sevoflurane yielded a decrease in resistive pressure (Fig. 1), probably due to an increase in the internal diameter of the central airways (Table 1 and Fig. 2C). This finding could be explained by both a neural reflex pathway and by a direct concentration-dependent bronchodilatory effect, which is partially epithelium-dependent (29). Moreover, we cannot exclude that the bronchodilation induced by sevoflurane depends on the production of nitric oxide (30). Wiklund et al. (31) studied the relaxation of airway smooth muscle by volatile anesthetics in isolated guinea pig bronchi and observed that sevoflurane relaxed airway smooth muscle contraction induced by leukotriene C4, an important mediator of asthmatic airway constriction.

Not only central airways but also distal airways and lung parenchyma are involved in the functional changes of asthma (11,12). We observed that lung viscoelastic/inhomogeneous pressure and static elastance diminished during sevoflurane anesthesia (Fig. 1) because of the dilation of distal airways and alveolar units. Indeed, we measured a smaller amount of alveolar collapse (Table 1 and Figs. 2C and D), indicating that sevoflurane can be useful to lessen inhomogeneities present in chronic asthma. In addition, airway dilation causes less distortion of the surrounding parenchyma, and this could result in a reduction in viscoelastic stress adaptation, which could also decrease ΔP_2.

As shown in Figure 1, the overall pressure (ΔP_{tot}) used to overcome resistive and viscoelastic elements (central and peripheral mechanical components, respectively) was smaller after sevoflurane anesthesia. This finding stems from a significant decrease in ΔP_1 and in ΔP_2. Our results are in agreement with previous studies conducted with induced bronchoconstriction, when a decline in total lung resistance was reported after sevoflurane administration in animals with normal lung reactivity (32–34). Similarly, Schutz et al. (35) administered volatile anesthetics in an animal model of bronchial hyperreactivity and observed that sevoflurane reversed bronchoconstriction transiently. This result was attributed to the acute epithelial damage induced by sensitization, which could interfere with the maintenance of the bronchodilator effect of the sevoflurane (29,35).

In normal humans, Rooke et al. (14) observed that sevoflurane decreased respiratory system resistance after tracheal intubation, and Goff et al. (36) reported that sevoflurane caused moderate bronchodilation when compared with sodium thiopental and desflurane. In contrast, Habre et al. (13) observed an increase in respiratory system resistance after endotracheal intubation in asthmatic children anesthetized with sevoflurane. Indeed, airway instrumentation is a major cause of bronchoconstriction mediated by the parasympathetic nervous system and by activation of the terminals of C-fibers afferents (37), even in nonasthmatic subjects. Hence, the increased lung resistance observed in their investigation should not be ascribed solely to sevoflurane. Furthermore, the comparison between our study and that of Habre et al. is unwarranted because, in the present study, respiratory mechanics were computed 15–20 min after tracheal intubation, and the measurements did not last longer than 30 min.

Correa et al. (24) analyzed lung mechanics and histology in normal rats anesthetized with sevoflurane and detected no changes in airway resistance. The authors reported an increase in ΔP_2 and E_{st}, as well as higher degrees of alveolar collapse and hyperinflation, allied to the presence of secretion in large and small airways. In contrast, in our study, sevoflurane anesthesia remarkably induced bronchodilation and decreased resistive pressure in normal mice. Besides, no difference in ΔP_2 and E_{st} was detected in normal animals, and there was no evidence of central or peripheral airway secretion, not even among ovalbumin-sensitized animals. These contrasting results could be attributed to the high amount of secretion in central airways in rats anesthetized with sevoflurane, probably preventing the observation of decreased airway resistance. Moreover, experimental conditions and the species analyzed were different in both studies. Patterns are unique not only for different species, but even among strains. Animals with different ages and diverse histological and subcellular structure of the airways can respond with a dissimilar airway behavior (20,38,39).

Even though our results cannot be extrapolated directly to humans, they suggest that sevoflurane may be advantageous in chronic asthma, because it acted not only on large airways, but on distal airways and lung parenchyma as well, decreasing lung inhomogeneities, even in a remodeled airway.

In conclusion, in the present experiment we observed that sevoflurane anesthesia in a murine model of chronic allergic asthma induced dilation in central and distal airways, yielding reduced resistive and viscoelastic/inhomogeneous pressures applied to the lung. The former finding was related to airway dilation, whereas the latter was supported by the histological demonstration of smaller areas of collapse of distal airspaces.

ACKNOWLEDGMENTS

We express our gratitude to Mr. Antonio Carlos de Souza Quaresma, Mr. Andre Benedito da Silva, and Mr. Sergio Cezar da Silva Junior for their skillful technical assistance.

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