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BRIEF REPORT

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Animal model of unilateral ventilator-induced lung injury

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Abstract *Objective:* To design, implement, and test a selective lung ventilator for setting a rat model of unilateral ventilator-induced lung injury (VILI). *Design and setting:* Interventional animal study in a university laboratory for animal research. *Subjects:* Anesthetized and paralyzed male Wistar rats. *Interventions:* A selective ventilator designed to apply varying tidal volume, PEEP, and breathing gas to each lung of the rat was implemented and evaluated. Five control animals were ventilated at 7 ml/kg (3.5 ml/kg each lung). Unilateral VILI was induced in six animals subjected to selective ventilation (3.5 ml/kg in one lung and 15 ml/kg in the other lung). After 3 h of ventilation the animals were killed and the lungs excised. *Measurements and results:* Lung edema was assessed by means of the ratio between wet and dry lung weights. No significant differences were found in lungs of control animals (5.16±0.22 and 4.96±0.25), but the W/D ratio in the over ventilated lung (8.98±3.80) was significantly greater than that in the normally ventilated lung (4.76± 0.15), indicating selective induction of lung edema by over stretch. *Conclusions:* This selective ventilator can be implemented into a rat model of unilateral VILI to gain further insight into the mechanisms of pulmonary injury induced by different ventilatory strategies

Keywords Mechanical ventilation · Lung injury · Lung edema · Lung overstretch · High volume ventilation · Selective lung ventilation

Introduction

Inadequate settings for administering mechanical ventilation can give rise to side effects such as lung edema [1], pulmonary, and systemic inflammation [2] or bacterial infectious dissemination [3]. Given its clinical relevance, ventilator-induced lung injury (VILI) has been the subject of considerable basic and clinical research [4, 5]. Animal models are most useful for investigating the pathophysiology of VILI and its still controversial mechanisms [6]. Most animal research on VILI has been carried out by comparing the results obtained in two groups of tracheally intubated animals: one group subjected to the investigated ventilatory strategy and the other to reference ventilation. To facilitate the experimental investigation of VILI the aim of this work was to develop and test the suitability of a novel animal model of unilateral VILI. The method was based on a selective ventilator allowing the simultaneous administration of reference ventilation to one lung and a different ventilation strategy to the other lung of the same animal [7].

Methods

Description of the selective ventilator

The selective ventilator was based on ventilating each lung with one independent unit. As shown in Fig. 1, each of these two ventilatory units was based on a servocontroled actuator (HS-325HB, Hitec, Powdy, Calif., USA) which moved the piston of a conven-

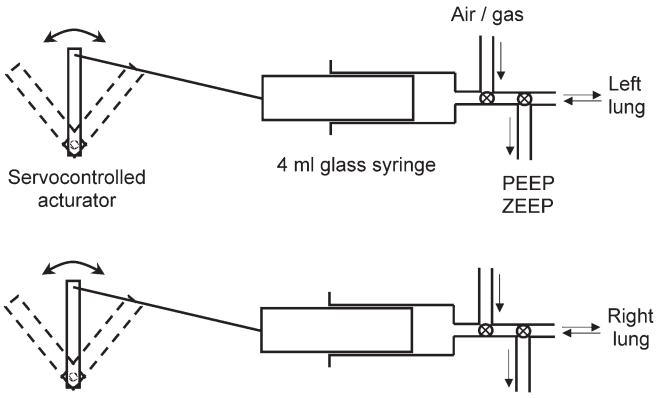

Fig. 1 Diagram of the selective ventilator implemented. Each lung was ventilated by means of an independent unit (see text for explanation)

tional glass syringe (5 ml, BD, Franklin Lakes, N.J., USA). The outlet of the syringe was connected to a three-way electrovalve (Z830A, Sirai, Bussero, Italy). This valve connected the syringe outlet to the atmosphere or to the inspiratory line. The expiratory line was connected to a two-way electrovalve (Z810A) which opened or closed the expiratory line (Fig. 1).

The actuator displacement (i.e., the displacement of the syringe piston) was electronically driven by a sinusoidal signal. During the inspiratory phase the expiratory valve was closed and the inspiratory valve connected the syringe to the inspiratory line. Accordingly, a sinusoidal tidal volume was fed to the lung. During expiration the inspiratory valve closed the inspiratory line and allowed the syringe to be filled with room air or with any other gas mixture. Connecting the expiratory line to a tube with its tip submerged in a given depth of water allowed the administration of positive end-expiratory pressure (PEEP).

Induction of unilateral VILI

The animal study, which was approved by the Ethics Committee for Animal Experimentation at the University of Barcelona, was conducted on 14 male Wistar rats weighing 250–300 g. The animals were intraperitoneally anesthetized with 100 mg thiobutabarbital per kilogram of body weight. A tracheotomy was performed and two cannulas (16GA BD Adsyte Pro, Becton Dickinson, Madrid, Spain) were introduced; one was placed in the right lung and the other in the left lung. After each lung was independently intubated, the animal was paralyzed by instillation of 0.4 mg/kg bw pancuronium bromide into the penile venous, and the intrabronchial cannulas were connected to the corresponding inspiratory and expiratory lines of the ventilator by means of a T-piece. The airway pressure was measured with a transducer placed at the T-piece. The expiratory flow of each lung was measured with two pneumotachographs placed at the corresponding expiratory lines of the ventilation circuit. The expired volumes were computed by integration of the expiratory flow signals. Possible air leaks were assessed by inspection of the expired tidal volumes. Anesthesia and paralysis were maintained by intermittent injections of thiobutabarbital and pancuronium bromide, respectively.

Unilateral VILI was induced in a group of six rats subjected to selective ventilation. One lung was normally ventilated with a tidal volume of 3.5 ml/kg bw (corresponding to a conventional total ventilation of 7 ml/kg bw). The other lung was overstretched with a tidal volume of 15 ml/kg bw (corresponding to a total ventilation of 30 ml/kg bw). In a control group of five rats the animals were conventionally ventilated by administering a tidal volume of 3.5 ml/kg bw to each lung. In all cases the rats were ventilated for 3 h (60 resp/min, PEEP=0).

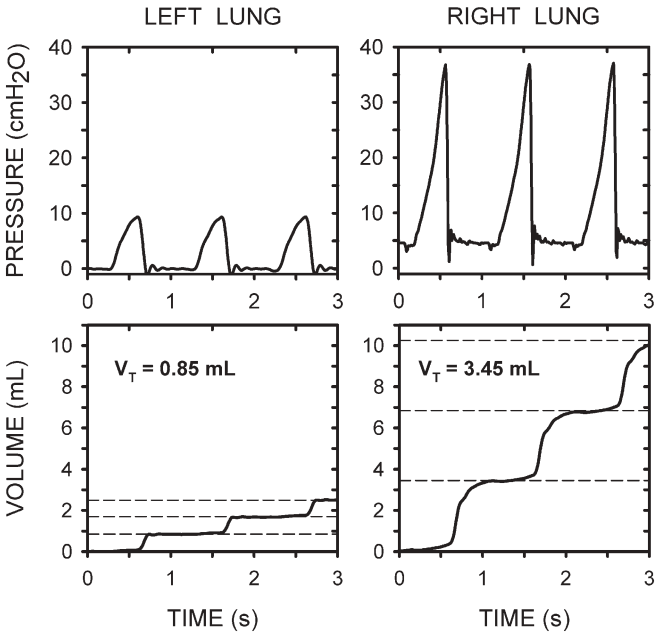

Fig. 2 Example of the pressure (*above*) and expiratory volume (*below*) measured at the airway opening in the left and right lungs of a rat ventilated with the differential ventilator designed. The volume is not reset to zero at the beginning of each inspiration because this signal is the direct integration of expiratory flow. *Dashed horizontal lines in the volume plots* correspond to the tidal volume in each breath. The left lung was ventilated with a normal tidal volume whereas the right lung was over stretched with a fourfold tidal volume and administration of 5 cmH_2O of PEEP

At the end of the 3-h period of ventilation the animals were killed by exsanguination through the abdominal aorta. The lungs were excised, and their large airways were removed. Each lung was enclosed in a sealed box and was frozen and stored at −80°C for subsequent analysis. At the end of the series of animal experiments lung edema was assessed by the ratio between the wet (W) and dry (D) weight of the lung [8]. To compute the W/D ratio the stored frozen lungs were defrosted at room temperature (4 h), weighed (W), heated at 50°C for 24 h and weighed again (D).

Data are shown as mean ±SD. Differences between the W/D ratios in the two lungs of each animal were assessed by Student's *t* test.

Results

The selective ventilator simultaneously administered different tidal volumes and PEEPs in each lung of the animals covering the ranges required for modeling injurious ventilation in rats. An example of the considerably asymmetrical ventilation that can be applied with the ventilator is shown in Fig. 2. The left lung of the rat was ventilated with a typical tidal volume of 0.85 ml with no PEEP. The right lung was overstretched by administering

a fourfold greater tidal volume (3.45 ml) and a PEEP of 5 cmH_2O. The peak pressures recorded at the airway opening of each lung were markedly different (9.5 cmH_2O vs. 37.5 cmH_2O, respectively).

Administration of selective ventilation with the ventilator designed allowed us to induce unilateral lung injury. In the rats with symmetrical ventilation with conventional tidal volume the two lungs exhibited virtually the same normal W/D ratio (5.16±0.22 and 4.96±0.25; p=0.21). By contrast, in the rats subjected to asymmetrical ventilation the lung ventilated with a conventional tidal volume showed a normal W/D ratio (4.76±0.15) whereas the overstretched lung exhibited a considerably increased W/D ratio of 8.98±3.80 (p=0.02), indicating lung edema [8].

Discussion

We describe a new animal model of unilateral VILI. The model is based on a selective ventilator able to administer different tidal volumes, PEEP levels, and breathing gases to each lung. To demonstrate its suitability for unilateral VILI studies lung edema by over stretch was selectively induced in a group of rats ventilated with a different tidal volume in each lung. The ventilator designed for selective ventilation is cheap and easy to implement given that it consists of only three general purpose components: conventional glass syringes, pinch solenoid valves, and servocontrolled position actuators commonly used for modeling. The signals driving the actuators and the synchronism signals to the electrovalves can be generated by an electronic circuit or by a personal computer equipped for instrumentation control (general purpose analog/digital output board and software).

The ventilator was implemented with two 5 ml syringes to cover the range of tidal volumes required in rats. However, using syringes with different volumes could allow the implementation of the unilateral VILI model for other rodent species. This could be achieved by simply adapting the gain of the signal driving the actuator. Although a sinusoidal volume pattern was used in this work, the ventilator can reproduce any inspiratory volume waveform and inspiratory/expiratory ratio. Given that the actuator employed incorporates the position (i.e., volume) servocontrol, modification of the ventilation pattern would only require replacing the sinusoidal driving signal by the desired volume ventilation waveform.

Selective ventilation requires independent intubation of the two main bronchi. In contrast to the case of tracheal intubation, it is not possible to bind the bronchial intubation area tightly to avoid leaks. Nevertheless, potential leaks can be easily quantified by measuring the expired tidal volume or indirectly assessed by inspection of the plateau of airway pressure after end-inspiratory airway occlusion. In the experiments leaks were found in 4 of the 28 intubated lungs (14 rats), and 3 animals were consequently discarded. These leaks could probably be prevented by using intubation cannulas with different diameters, as in the clinical setting of neonatal mechanical ventilation where uncuffed cannulas are also used for intubation.

In conclusion, the novel animal model of VILI proposed in this work is simple and easy to implement. The selective ventilator designed and tested in rats could be used in other rodent species after only minor modifications. One notable advantage of this model for studying VILI is that biological variability is reduced since the same animal is used as test and control. This reduction in biological variability is expected to be particularly useful in animals with previously induced inflammation or infection since the magnitude of these experimental interventions is difficult to control. Moreover, selective ventilation can be used to investigate VILI under conditions where the ventilatory strategy under test compromises the physiological status or the viability of the animal. For instance, during extreme hypoxia/hyperoxia [9, 10] and/or hypercapnia/hypocapnia [11, 12] or when studying the effects of inspiratory frequency [13, 14]. In this cases one lung can be subjected to the VILI test while the other lung is ventilated to maintain blood gases within a safe range.

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