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Original article

Improvement in ARDS experimental model installation: Low mortality rate and maintenance of hemodynamic stability

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ABSTRACT

Introduction: Many experimental models using lung lavage have been developed for the study of acute respiratory distress syndrome (ARDS). The original technique has been modified by many authors, resulting in difficulties with reproducibility. There is insufficient detail on the lung injury models used, including hemodynamic stability during animal preparation and drawbacks encountered such as mortality. The authors studied the effects of the pulmonary recruitment and the use of fixed tidal volume (Vt) or fixed inspiratory pressure in the experimental ARDS model installation.
Methods: Adult rabbits were submitted to repeated lung lavages with 30 ml/kg warm saline until the ARDS definition ($PaO_2/FiO_2 \leq 100$) was reached. The animals were divided into three groups, according to the technique used for mechanical ventilation: 1) fixed Vt of 10 ml/kg; 2) fixed inspiratory pressure (IP) with a tidal volume of 10 ml/kg prior to the first lung lavage; and 3) fixed Vt of 10 ml/kg with pulmonary recruitment before the first lavage.
Results: The use of alveolar recruitment maneuvers, and the use of a fixed Vt or IP between the lung lavages did not change the number of lung lavages necessary to obtain the experimental model of ARDS or the hemodynamic stability of the animals during the procedure. A trend was observed toward an increased mortality rate with the recruitment maneuver and with the use of a fixed IP.
Discussion: There were no differences between the three study groups, with no disadvantage in method of lung recruitment, either fixed tidal volume or fixed inspiratory pressure, regarding the number of lung lavages necessary to obtain the ARDS animal model. Furthermore, the three different procedures resulted in good hemodynamic stability of the animals, and low mortality rate.

1. Introduction

Acute respiratory distress syndrome (ARDS) is characterized by inflammation, pulmonary edema, and hypoxemia, and is associated with a variety of etiologies (Ashbaugh, Bigelow, Petty, & Levine, 1967). Because ARDS carries high morbidity, mortality, and financial cost, many efforts have been made to study its pathophysiology (Ashbaugh et al., 1967), treatment (Rice & Bernard, 2006), lung protective ventilation strategies (Amatto et al., 1998), and surfactant treatment (Perez-Benavides, Riff, & Franks, 1995).

Several ARDS animal models have been described (Matute-Bello, Frevert, & Martin, 2008), including lung lavage with NaCl 0.9% (saline) (Lachmann, Robertson, & Vogel, 1980), injection of oleic acid (Cakar, der Kloot, Youngblood, Adams, & Nahum, 2000; Schuster, 1994), intratracheal bleomycin infusion (Olman, Mackman, Gladson, Moser, & Loskutoff, 1995), intratracheal instillation of infant formula or human breast milk (Nishina, Mikawa, Takao, Maekawa, & Obara, 1999), subcutaneous administration of N-nitroso-N-methylurethane (Kerr, Veldhuizen, & Lewis, 2001; Lewis, Ikegami, & Jobe, 1992), hydrochloric acid aspiration (Nishina et al., 1998), or using intratracheal instillation of lipopolysaccharide (Sato et al., 2002).

One widely used ARDS animal model was originally developed by Lachmann et al. (1980). In this model lung injury is caused by successive lavages with warm saline (Lachmann et al., 1980). Subsequently several studies have been published using the model introduced by Lachmann but with several modifications (Barsotti, Chundu, Silvka, Sephus, & Hallman, 1996; Hickling et al., 1996; Rotta, Gunnarsson, Fuhman, Hernan, & Steinhorn, 2001), which might affect the reproducibility and heterogeneity of these studies. However, neither Lachmann in his original report nor any of the other authors described all the details necessary to reproduce the lung injury model used, especially the variations in hemodynamic stability during the animal preparation

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and drawbacks encountered such as mortality (Lachmann et al., 1980). In fact, when we tried to reproduce some of the model establishment conditions described in the literature, we observed high animal mortality rates. Indeed, Lachmann mentioned that features considered for establishment of the model influence the survival of the animals during and after the experimental procedure. Therefore, it is possible that the different ARDS animal model establishment methods described in the literature may have compromised comparison of results and possibly affected the choice of interventions for treatment of this syndrome. Hence, the aim of this study was to improve the ARDS animal model which is already widely used – the Lachmann model – to create a version that is easy to reproduce, maintains the animal at a hemodynamic stable condition, and with low mortality rates, while still replicating the human ARDS lung condition. We think that clear and reproducible model establishment conditions may help advance our knowledge on treatment solutions for this disease.

2. Methods

This study was conducted at the Experimental Research Unit, Department of Pediatrics of the University of Sao Paulo, Brazil. The study protocol was approved by the Commission on Ethics for Analysis of Research Projects of the University of Sao Paulo. The established protocol for management of laboratory animals was followed.

2.1. Animal preparation

A total of thirty-one adult New Zealand White rabbits weighing an average of 3.125 g were used in this protocol and prepared according to Rossi et al. (Rossi et al., 2008). Briefly, animals were sedated (through an intramuscular injection of 10 mg/kg ketamine and 0.1 mg/kg acepromazine) and the anterior region of their neck was anesthetized (subcutaneous injection of 2% xylocaine), before dissection and insertion of 4Fr catheters in the carotid (for monitoring blood pressure, heart rate, and arterial gasometry) and in the jugular vein (for continuous infusion of 5% dextrose solution at 4 ml/kg/h). Next, the trachea was exposed and an endotracheal tube (3.5 mm or 4.0 mm internal diameter) was passed through and tied. Blood pressure, heart rate, and body temperature were continuously monitored. Body temperature was maintained between 38 °C and 39.5 °C using warming pads. During the whole study the animals remained in a dorsal decubitus position.

2.2. Initial ventilation and respiratory monitoring

Animals were initially ventilated (Inter 3®, Intermed, Sao Paulo, Brazil) with an FiO_2 of 1.0, tidal volume of 10 ml/kg, positive end-expiratory pressure (PEEP) of 5 cm H_2O, respiratory rate of 50 breaths/min, inspiratory time of 0.5 s, and flow of 8 l/min. Inspiratory pressure was recorded through a pressure transducer (Validyne®, model DP45-24), connected to software developed for respiratory mechanical analysis (LabVIEW 5.1®, National Instruments, Austin, USA and R. A. Eletro Sistemas LTDA, Campinas, Brazil). Tidal volume was evaluated through the integration of the time and flow signals obtained from a pneumotachograph (Model 8431 series – Hans Rudolph® Inc., Kansas City, USA) also connected to a pressure transducer (Validyne®, model DP45 – 14), and connected to the same data acquisition software described above. As soon as the ventilation started, the animals received pancuronium bromide 0.1 mg/kg intravenously to obtain muscle paralysis. The pancuronium bromide dose was repeated every time a spontaneous respiratory effort was observed.

2.3. Induction of lung injury

Animals were divided into three study groups, according to the variation of the original method used for ARDS installation: Group 1: fixed tidal volume (Vt) of 10 ml/kg throughout the procedure, with no previous pulmonary recruitment; Group 2: fixed inspiratory pressure (IP) throughout the procedure, defined as the inspiratory pressure needed to obtain a tidal volume of 10 ml/kg prior to the first lung lavage, with no previous pulmonary recruitment; Group 3: fixed tidal volume (Vt) of 10 ml/kg throughout the procedure with pulmonary recruitment before the first lavage. Prior to this, we performed a pilot study in which it was determined that the lower inflection point (Pflex) of the volume–pressure curve in healthy lungs was 8 cm H_2O. Therefore, the pulmonary recruitment in Group 3 was performed with PEEP of 10 cm H_2O (Pflex + 2 cm H_2O), defined by a pilot study, for 5 min with Vt of 6 ml/kg and FiO_2 of 1.0. Subsequently, the ventilator parameters were set as the IP needed to obtain a Vt of 10 ml/kg and PEEP of 5 cm H_2O throughout the procedure.

The remaining procedure for lung injury was induced according to the method originally described by Lachmann (Lachmann et al., 1980). Briefly, successive lung lavages were performed using 30 ml/kg aliquots of saline at 37 °C. Before the lavages the animal was disconnected from the ventilator and then warmed saline was introduced by gravity associated with thoracic massage, through the endotracheal tube, at a maximal pressure of 40 cm H_2O, and drained by gravity aided by thoracic massage in an attempt to remove as much fluid from the lungs as possible. The total time for each lung lavage was 60 s (20 s for infusion and 40 s for draining). This procedure was performed as many times as necessary at 5 min intervals, until the ARDS model was reached, defined as $PaO_2/FiO_2 \leq 100$ determined by arterial gasometry. To confirm the establishment of the ARDS model, a new arterial gasometry measurement was obtained after 10 min of stabilization following the last lung lavage. Every time this parameter was not reached the lung lavage procedure was repeated. Heart rate and arterial blood pressure were continuously monitored and were recorded before each lung lavage.

To monitor the blood gases, arterial samples were read immediately before and after each lung lavage with the help of I-Stat Corporation® equipment (Windsor, USA).

The efficiency of each method to study lung lavage was determined by calculating the volume percentage of fluid recovery, the record of the volume infused, and the volume removed in each aliquot.

After confirmation of the installation of the ARDS model the animals were deeply sedated with sodium pentobarbital (25 mg/kg, IV), followed by sacrifice via severing the abdominal aorta.

2.4. Statistical analysis

Quantitative data were compared by one-way analysis of variance, and Student–Newman–Keuls test was used as a post-hoc test whenever necessary. Categorical data were examined with Chi-square or Fisher's exact test, as indicated. Statistical significance was set at $p<0.05$.

The number of animals needed for the study considering the average number of lung lavages required to install the ARDS model with $\alpha = 0.05$ and a test power of 0.80, was 9 animals per group. This sample size was necessary to detect a difference between the study groups of about one lavage. The sample size parameter used was the same as published elsewhere (Kerr et al., 2001).

3. Results

Thirty-one animals were distributed among three study groups that shared the same lung lavage method (temperature, time, and volume of saline), but that varied according to the ventilation procedure and the use of pulmonary recruitment.

The first condition we sought to establish was the ideal number of lung lavages required to obtain the ARDS model (Table 1) according to the ventilation method and the lung recruitment. We found that, among the three study groups, only a small number of lavages were required to establish a PaO_2/FiO_2 ratio lower than 100. To enhance