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Effects of short-term propofol and dexmedetomidine on pulmonary morphofunction and biological markers in experimental mild acute lung injury

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ABSTRACT

We evaluated whether the short-term use of dexmedetomidine and propofol may attenuate inflammatory response and improve lung morphofunction in experimental acute lung injury (ALI). Thirty-six Wistar rats were randomly divided into five groups. Control (C) and ALI animals received sterile saline solution and *Escherichia coli* lipopolysaccharide by intraperitoneal injection respectively. After 24 h, ALI animals were randomly treated with dexmedetomidine, propofol, or thiopental sodium for 1 h. Propofol reduced static lung elastance and resistive pressure and was associated with less alveolar collapse compared to thiopental sodium and dexmedetomidine. Dexmedetomidine improved oxygenation, but did not modify lung mechanics or histology. Propofol was associated with lower IL (interleukin)-6 and IL-1β expression, whereas dexmedetomidine led to reduced inducible nitric oxide (iNOS) and increased nuclear factor erythroid 2-related factor 2 (Nrf2) expression in lung tissue compared to thiopental sodium. In conclusion, in this model of mild ALI, short-term use of dexmedetomidine and propofol led to different functional effects and activation of biological markers associated with pulmonary inflammation.

1. Introduction

Dexmedetomidine and propofol have been used for sedation and short-term surgical procedures in patients with acute respiratory distress syndrome (Choi, 2013; Iapichino et al., 2003; Jin et al., 2013). Dexmedetomidine has been reported to decrease inflammatory response (Gu et al., 2011; Yang et al., 2009) through a reduction in oxidative stress (Yang et al., 2011, 2008), whereas propofol reduces pulmonary inflammation (Chen et al., 2008; Yeh et al., 2011), oxidative stress (Chen et al., 2008; Tsao et al., 2008), and apoptosis (Kalimeris et al., 2011). The immunomodulatory capacity of these agents has been investigated before (Gao et al., 2010; Xu et al., 2013), at the moment of lung injury induction (Gu et al., 2011; Yeh et al., 2011) and during long-term use (Chu et al., 2007; Yang et al., 2008). Furthermore, their effects on lung inflammation depend on timing (Chen et al., 2008; Taniguchi et al., 2008) and length (Gao et al., 2010) of administration. So far, no study has evaluated their impact on lung morphofunction and molecular biology during short-term use and after the establishment of acute lung injury.

The aim of the present study was to analyze the effects of dexmedetomidine and propofol on lung mechanics, histology, and biological markers associated with pro- and anti-inflammatory activities, fibrogenesis, and oxidative stress in an experimental model of mild acute lung injury during controlled mechanical ventilation in rats.

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2. Material and methods

This study was approved by the Research Ethics Committee of the Federal University of Rio de Janeiro Health Sciences Centre (CEUA 019). 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 U.S. National Academy of Sciences.

2.1. Animal preparation and experimental protocol

Thirty-six female Wistar rats (weight 250–300 g) were used for this study. Thirty rats received *Escherichia coli* lipopolysaccharide (LPS O55:B5), 1000 μg suspended in saline solution to a total volume of 1000 μl, by intraperitoneal injection (i.p.) (acute lung injury, ALI). Six rats received saline solution alone (1000 μl, i.p.) and constituted the control (C) group. Of the 30 ALI animals, 24 were allocated across three subgroups ($n = 8$/subgroup). These animals received thiopental sodium (50 mg/kg, i.p.), underwent tail vein cannulation with a 24-gauge polyethylene catheter, and were then randomly assigned to receive: (a) thiopental sodium, 5 mg/kg/h by intravenous infusion; (b) dexmedetomidine, one 5-μg bolus over 10 min followed by intravenous infusion at a rate of 0.5 μg/kg/h; or (c) propofol by intravenous infusion, at a rate of 100–200 μg/kg/min for 10 min and 75–100 μg/kg/min thereafter. The infusion rates of propofol and dexmedetomidine were based on previous studies that reported a negligible effect of these anesthetic agents on hemodynamic parameters in septic animals (Chu et al., 2007; Gu et al., 2011; Song et al., 2009; Yang et al., 2008). Additionally, these infusion rates were adopted to ensure a similar level of anesthesia in all groups. Finally, the whole saline control (C) group ($n = 6$) and the remaining ALI animals not assigned to any of the three treatment subgroups ($n = 6$) received thiopental sodium (50 mg/kg i.p.) and were assigned to non-ventilated (NV) groups (C-NV and ALI-NV, respectively) for lung morphometry and molecular biology analysis. C-NV and ALI-NV rats were euthanized 5 min after administration of thiopental sodium so that this anesthetic agent would not affect molecular biology parameters.

During spontaneous breathing, the level of anesthesia was assessed by evaluating pupil size, position, and response to light, position of the nictitating membrane, and movement in response to tail stimulation. When an appropriate plane of anesthesia had been achieved, tracheotomy was performed after local anesthesia (lidocaine 1%, subcutaneously). A polyethylene catheter (PE-10) was inserted into the carotid artery for blood sampling and monitoring of mean arterial pressure (MAP). Electrocardiogram (EKG) tracings, MAP, and rectal temperature were continuously recorded (Networked Multi-Parameter Veterinary Monitor LifeWindow™ 6000V, Digicare Animal Health, Florida, USA). Gelafundin® (B. Braun, Melsungen, Germany) was administered (titrated in 0.5 ml increments), if necessary, to maintain MAP ≥70 mmHg. Pancuronium bromide was then administered (2 mg/kg in the tail vein) to maintain paralysis for 1 h, with an additional bolus equal to half of the initial dose whenever necessary. Lungs were mechanically ventilated (Servo-i, MAQUET, Solna, Sweden) in volume-controlled mode with tidal volume (V_T) = 6 ml/kg, respiratory rate (RR) = 80 breaths/min, fraction of inspired oxygen (FIO_2) = 0.4, and zero end-expiratory pressure (ZEEP) during 5 min. Functional data (EKG, MAP, mechanics, and rectal temperature) had been collected (Baseline-ZEEP) to evaluate whether lung mechanical impairment was similar in all ALI groups at the beginning of the experiment. Positive end-expiratory pressure (PEEP) was elevated to 3 cmH_2O, and functional data measurement repeated. Arterial blood (300 μl) was drawn into a heparinized syringe to determine arterial oxygen partial pressure (PaO_2) (i-STAT, Abbott Laboratories, Illinois, USA) (Baseline-PEEP). Lung mechanics and PaO_2 were measured after 1 h of mechanical ventilation (End-PEEP). Animals were then euthanized and lungs extracted for histological and molecular biology analysis.

2.2. Data acquisition and processing

Airflow, airway (P,aw), and esophageal pressures (Pes) were continuously recorded throughout the experiments with a computer running custom software written in LabVIEW® (National Instruments; Austin, Texas, USA). Tidal volume (V_T) was calculated by digital integration of the flow signal. Transpulmonary pressure (P,L) was calculated during inspiration and expiration as the difference between P,aw and Pes. All signals were filtered (200 Hz), amplified in a 4-channel signal conditioner (SC-24, SCIREQ, Montreal, Quebec, Canada), and sampled at 200 Hz with a 12-bit analogue-to-digital converter (National Instruments; Austin, Texas, USA). Static lung elastance (Est,L) and resistive pressure (ΔP1,L) were computed offline by a routine written in MATLAB (Version R2007a; The Mathworks Inc, Natik, Massachusetts, USA) (Santos et al., 2012).

2.3. Lung histology

A laparotomy was performed and heparin (1000 IU) injected into the tail vein. The trachea was clamped at PEEP = 3 cmH_2O and animals were euthanized by severing the abdominal aorta and vena cava, producing massive hemorrhage and rapid death by exsanguination. Lungs were removed *en bloc* at the same PEEP level in both groups to avoid distortion of lung morphometry. The right lung was fixed in 3% buffered formaldehyde and embedded in paraffin. Slices (4 μm thick) were cut and stained with hematoxylin-eosin.

Lung morphometry was assessed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (OlympusBX51, Olympus Latin America-Inc., Brazil). The volume fractions of the lung occupied by hyperinflated structures (alveolar ducts, alveolar sacs, or alveoli wider than 120 μm), collapsed alveoli (alveoli with rough or plicate walls), and normal pulmonary areas (those not presenting overdistended or plicate walls) were determined by the point-counting technique at a magnification of ×200 across 10 random, noncoincident microscopic fields (Weibel, 1990). To quantify interstitial edema, five arteries were transversely sectioned. The number of points falling on areas of perivascular edema and the number of intercepts between the lines of the integrating eyepiece and the basal membrane of the vessels were counted. The interstitial perivascular edema index was calculated as follows: number of points$^{1/2}$/number of intercepts (Santiago et al., 2010).

2.4. Biological markers of pro-inflammatory and anti-inflammatory activity, apoptosis, fibrogenesis, oxidative stress, and lung epithelial and endothelial cell damage

Quantitative real-time reverse transcription polymerase chain reaction (PCR) was performed to measure biological markers associated with inflammation (interleukin [IL]-6 and IL-1β), and oxidative stress (inducible nitric oxide synthase [iNOS], endothelial nitric-oxide synthase [eNOS], and nuclear factor erythroid 2-related factor 2 [Nrf2]). Central slices of the left lung were cut, collected in cryotubes, flash-frozen by immersion in liquid nitrogen, and stored at −80 °C. Total RNA was extracted using the SV Total RNA Isolation System (Promega Corporation, Fitchburg, Wisconsin, USA), following manufacturer recommendations. RNA concentration was measured by spectrophotometry in Nanodrop ND-1000. First-strand cDNA was synthesized from total RNA using the GoTaq® 2-STEP RT qPCR System (Promega