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Interactive effects of mechanical ventilation, inhaled nitric oxide and oxidative stress in acute lung injury

Carlos Fernando Ronchi[b,c,d,*], Ana Lucia Anjos Ferreira[a], Fabio Joly Campos[b], Cilmery Suemi Kurokawa[b], Mario Ferreira Carpi[b], Marcos Aurélio Moraes[b], Rossano Cesar Bonatto[b], Kyung-Jin Yeum[c], Jose Roberto Fioretto[b]

[a] Department of Internal Medicine, Botucatu Medical School – Sao Paulo State University (UNESP), Botucatu, SP, Brazil
[b] Department of Pediatrics, Botucatu Medical School – Sao Paulo State University (UNESP), Botucatu, SP, Brazil
[c] Jean Mayer USDA – Human Nutrition Research Center on Aging, Tufts University, Boston, MA, USA
[d] Department of Health Sciences – Universidade do Sagrado Coração (USC), Bauru, SP, Brazil

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ABSTRACT

To compare conventional mechanical ventilation (CMV) and high-frequency oscillatory ventilation (HFOV), with/without inhaled nitric oxide (iNO), for oxygenation, inflammation, antioxidant/oxidative stress status, and DNA damage in a model of acute lung injury (ALI). Lung injury was induced by tracheal infusion of warm saline. Rabbits were ventilated at F_{IO_2} 1.0 and randomly assigned to one of five groups. Overall antioxidant defense/oxidative stress was assessed by total antioxidant performance assay, and DNA damage by comet assay. Ventilatory and hemodynamic parameters were recorded every 30 min for 4 h. ALI groups showed worse oxygenation than controls after lung injury. After 4 h of mechanical ventilation, HFOV groups presented significant improvements in oxygenation. HFOV with and without iNO, and CMV with iNO showed significantly increased antioxidant defense and reduced DNA damage than CMV without iNO. Inhaled nitric oxide did not beneficially affect HFOV in relation to antioxidant defense/oxidative stress and pulmonary DNA damage. Overall, lung injury was reduced using HFOV or CMV with iNO.

1. Introduction

Acute respiratory distress syndrome (ARDS) is characterized by an inflammatory process that causes disruption of the alveolar-capillary barrier leading to interstitial and alveolar edema. There is also an increase in pulmonary vascular resistance, which has a rapid onset and persists even after hypoxia correction (Phua and Govert, 2008). According to the new Berlin definition, mild hypoxemia is characterized as 200 mmHg $< Pa_{O_2}/F_{IO_2} \leq$ 300 mmHg with PEEP or CPAP $\geq$ 5 cmH_2O; moderate hypoxemia as 100 mmHg $< Pa_{O_2}/F_{IO_2} \leq$ 200 mmHg with PEEP $\geq$ 5 cmH_2O; and severe hypoxemia as $Pa_{O_2}/F_{IO_2} \leq$ 100 mmHg with PEEP $\geq$ 5 cmH_2O (ARDS: The Berlin definition, 2012).

Although mechanical ventilation (MV) is an important support for patients with ARDS, it can cause additional lung injury (Dahlem et al., 2007) due to the large inspired tidal volume (V_T) or high ventilator pressures (Ware and Matthay, 2000). Protective conventional mechanical ventilation has shown decreased mortality rates in clinical studies (ARDS Network, 2000). High-frequency oscillatory ventilation (HFOV) is considered an attractive ventilatory mode of pulmonary protection (Allardet-Servent et al., 2008; Girard and Bernard, 2007). It uses a lower V_T (1–3 ml/kg) at a frequency well above normal physiological breathing (5–10 Hz) thus avoiding the larger alveolar pressure and volume excursions typical of conventional mechanical ventilation (CMV). In recent studies, we demonstrated that HFOV improved oxygenation, reduced inflammatory process and histopathological damage, and attenuated oxidative lung injury compared to CMV in an experimental acute lung injury (ALI) model (Ronchi et al., 2011, 2012).

The adjunctive use of inhaled nitric oxide (iNO), a potent endogenous vasodilator (Kohelet, 2003) has potential benefits (Heussel et al., 2003) without causing systemic vasodilatation and hypotension (Ichinose et al., 2004). However, its effect on pulmonary inflammatory response is still controversial, showing either increased (Sittipunt et al., 2001) or decreased (Bloomfield et al., 1997) release of inflammatory mediators. Nitric oxide is a reactive molecule produced by nitric oxide synthase (NOS) enzymes in a variety of cells. It is a free oxygen radical that can react with molecules to form toxic chemical compounds in the lung, including peroxynitrite, which damages DNA, induces lipid

* Corresponding author at: Health Science Department, Universidade do Sagrado Coração – USC, Bauru, SP 17011-160, Brazil. Tel.: +55 14 2107 7000; fax: +55 14 2107 7000.
E-mail address: ronchi.carlos@yahoo.com (C.F. Ronchi).

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peroxidation, and reacts with proteins, leading cellular apoptosis (Crow and Beckman, 1995). Nitric oxide inhibits adhesion molecule expression, inflammatory cytokine and chemokine, such as IL-8, MCP-1, among others (Guzik et al., 2003). Also, it can react with biologic targets directly or indirectly via formation of reactive intermediate products. Under aerobic conditions, the gas reacts with oxygen and superoxide radicals to form nitrogen dioxide and peroxynitrite, respectively. These reactive nitrogen species are capable of causing damage to macromolecules by oxidation of redox-active complexes or nitration of aromatic amines (Sittipunt et al., 2001).

These effects are mainly related to iNO dose, local oxidation–reduction potential, other inflammatory mediators, and oxygen-derived free radicals (Laroux et al., 2001). Our group recently showed that the early use of low dose iNO attenuated oxidative stress, and histopathological and inflammatory lung injury (Fioretto et al., 2012).

Currently, no study has compared the effects of HFOV and CMV together with iNO on lung oxidative stress and DNA damage using total antioxidant performance and comet assay, respectively.

Based on our previous results, we hypothesized that HFOV with iNO attenuates pulmonary oxidative stress in ALI. This study aimed to compare HFOV and CMV, both with and without iNO, for oxygenation, inflammation, histopathology, antioxidant defense, and DNA damage in an ALI model.

2. Methods

2.1. Design, animals, and instrumentation

A prospective, sham controlled animal study was conducted. Forty male 2.0–3.0 kg Norfolk white rabbits were anesthetized with ketamine (50 mg/kg intramuscularly) and xylazine (2 mg/kg intramuscularly) and instrumented as previously described (Fioretto et al., 2012; Ronchi et al., 2011, 2012). Briefly, rabbits were preoxygenated during spontaneous breathing with 100% oxygen by nose catheter. A tracheotomy was performed by inserting a tracheal tube (3.0–3.5 mm internal diameter; Portex, Hythe, UK) and securing it in position with umbilical tape. Ventilation was then immediately initiated with a Galileo Gold ventilator (Hamilton Medical AG, Sweden) in pressure-regulated-volume control mode with the following initial parameters: $F_{IO_2} = 1.0$; $V_T = 6$ ml/kg; PEEP = 5 cmH_2O; respiratory frequency (fR) = 40–50 breaths/min. These settings were maintained 15 min for stabilization. Once tracheotomy had been performed, a vascular catheter was inserted into the common carotid artery (22 Gauge Jelco, Introcan® Safety™ – B-Braun, Melsungen, Germany), and a double lumen catheter (5^{Fr} – Arrow International Inc., Reading, Philadelphia, USA) was advanced into the superior vena cava through the jugular vein. The arterial catheter was used to assess blood gases and arterial blood pressures using a monitoring system (LogiCal® da Medex, Dublin, USA) connected to a conventional physiological monitor (Dixtal 2010, Manaus, Brazil). The double lumen catheter was used to provide continuous infusion of sedatives, maintenance fluids, and vasoactive drugs.

Anesthesia was maintained with continuous intravenous infusion of ketamine (10 mg/kg/h). Muscle paralysis was induced by intravenous administration of pancuronium bromide (0.2 mg/kg) and maintained with 0.1 mg/kg doses as needed to control movement. If mean arterial pressure fell below 50 mmHg during the experiment, intravenous noradrenaline infusion (0.5–1 μg/kg/min) was initiated. The need for inotropic support was measured by using a vasoactive-inotropic score (Gaies et al., 2010). Maintenance fluid was provided by continuous infusion of 0.9% saline solution containing 5% dextrose at 4 ml/kg/h. Core temperature was monitored continuously by esophageal probe and body temperature maintained at 38–39 °C with electric warming pads. Continuous pulse oximetry was performed with the probe placed on a shaved portion of the rabbit's thigh.

Rabbits were cared for minimizing discomfort, distress, and pain in accordance with National Institute of Health guidelines. This study was approved by the Experimental Research and Ethics Committee, Botucatu Medical School, Sao Paulo State University and the Jean Mayer USDA – Human Nutrition Research Center on Aging at Tufts University, Boston, USA.

2.2. Lung injury induction

Lung injury was induced by lung lavage with 30 ml/kg aliquots of 0.9% warm saline solution (38 °C) as previously described (Fioretto et al., 2012; Ronchi et al., 2011, 2012). After stabilization, an arterial blood gas sample was obtained to verify that animals were hypoxemic (two values of $Pa_{O_2} \leq 200$ mmHg 15 min apart). After the stabilization period, animals were given two 30 s dynamic sustained inflations with a Paw of 30 cmH_2O to promote lung recruitment and equalize volume history (Mcculloch et al., 1988).

2.3. Experimental groups

Animals ($n = 40$) were assigned to one of five groups: (a) sham control [CG, $n = 8$: V_T 6 ml/kg, PEEP 5 cmH_2O]; (b) ALI + CMV [MVG, $n = 8$: V_T 6 ml/kg, PEEP 10 cmH_2O, Plateau pressure limited to $\leq$30 cmH_2O]; (c) ALI + CMV + iNO [MVGNO, $n = 8$: V_T 6 ml/kg, PEEP 10 cmH_2O, iNO 5 ppm, Plateau pressure limited to $\leq$30 cmH_2O, in a Galileo Gold ventilator (Hamilton Medical AG, Sweden)]; (d) ALI + HFOV [HFG, $n = 8$: Paw 12–14 cmH_2O, respiratory frequency (fR) 10 Hz, inspiratory time 33%, and initial pressure amplitude 20 cmH_2O]; and (e) ALI + HFOV + iNO [HFGNO, $n = 8$: Paw 12–14 cmH_2O, fR 10 Hz, inspiratory time 33%, iNO 5 ppm, and initial pressure amplitude 20 cmH_2O, in a SensorMedics 3100A ventilator (Viasys Healthcare, Yorba Linda, USA)]. In CMV mode, respiratory frequency was maintained at 40–50 breaths/min to reach targeted P_{CO_2} (40–45 mmHg); pressure amplitude in HFOV was modified to achieve the same P_{CO_2} level. F_{IO_2} was maintained at 1.0 throughout the experiment.

Arterial blood gas was obtained before (baseline) and after lung injury induction and every 30 min until 4 h observation was completed; analysis was by an ABL-3 blood gas analyzer (Bayer, Rapid Lab, Khiron 865).

2.4. Inhaled nitric oxide administration

Inhaled nitric oxide administration followed previously described guidelines and techniques (Fioretto et al., 2012, 2004). Briefly, NO blended with nitrogen was obtained from 20 L tanks connected to a pressure regulator (White Martins Gases Industriais – Praxair, Rio de Janeiro, Brazil). Tank concentrations were certified by the suppliers as 300 ppm nitric oxide in nitrogen. NO was continuously delivered to the rabbits via flowmeter directly into the inspiratory limb of the ventilator circuit, distal to the humidifier from a point 30 cm distal to the rabbit's tracheal tube. Inhaled nitric oxide and nitric dioxide (NO_2) concentrations were measured using an electrochemical sensor (JP Moryia Ind & Com Ltd., São Paulo, Brazil) from samples of circuit gas obtained as close as possible to the tracheal tube via a Y-piece. The NO/NO_2 electrochemical sensor was calibrated before use every day. Audio-visual alarms were calibrated at 1 ppm above administered iNO dose and at a maximum NO_2 concentration of 3 ppm. The delivery system was flushed thoroughly before use. In high-frequency oscillatory ventilation, iNO was delivered and monitored using a Pulmonox Mini (Namu, Messer, Griesheim, Austria).