Experimental Gerontology 96 (2017) 146–154

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Middle age exacerbates acute respiratory distress syndrome in a double hit murine model

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ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form 21 February 2017
Accepted 28 June 2017
Available online 1 July 2017

Section Editor: Richard Aspinall

Keywords:
Aging
Acute respiratory distress syndrome
Mechanical ventilation
Pulmonary vascular dysfunction
Lung edema

ABSTRACT

Rationale: In a recent systematic review, aging has been identified as the only factor independently associated with mortality during human acute respiratory distress syndrome (ARDS). We explored this age-dependent severity in a clinically relevant double hit murine ARDS model.
Methods: Young adult (Y, 10–12 weeks) and middle-old (O, 12–13 months) male C57BL6 mice underwent an aspiration of *Escherichia coli* lipopolysaccharide (LPS) or control saline vehicle. Twenty hours later, four groups of mice were sacrificed [$Y_{(control)}$, $O_{(control)}$, $Y_{(LPS)}$ and $O_{(LPS)}$]. Four other groups of mice underwent 3 h of low tidal volume (8 mL/kg) mechanical ventilation (MV) [$Y_{(MV)}$, $O_{(MV)}$, $Y_{(LPS\ +\ MV)}$ and $O_{(LPS\ +\ MV)}$]. Lung mechanics were assessed hourly during MV. Right ventricular pressure and cardiac output were measured at the end of the MV. After sacrifice, lung inflammation, edema and injury were explored with bronchoalveolar lavage (BAL) and histology.
Results: After saline aspiration, middle-old mice had a higher respiratory system compliance than young adult mice. LPS aspiration dramatically altered the baseline compliance in middle-old ($O_{(LPS)}$), but not in young adult ($Y_{(LPS)}$) mice. Middle-old mice had a more pronounced alteration in lungs mechanics during MV as compared to young adult mice. Lung inflammation (as assessed by the total cell count, IL-6, TNFα and MIP-2 concentrations in BAL fluid), systemic inflammation (as assessed by plasma IL-6 concentration) and alveolocapillary leak (as assessed by the total protein concentration of BAL fluid) were higher in $O_{(LPS)}$ and $O_{(LPS\ +\ MV)}$ mice as compared to $Y_{(LPS)}$ and $Y_{(LPS\ +\ MV)}$ mice, respectively. The combination of LPS + MV induced a higher lung injury as compared to LPS alone in middle-old mice but not in young adult mice. Hemodynamics (systemic blood pressure, cardiac output and pulmonary vascular resistances) were similar between $Y_{(MV)}$ and $O_{(MV)}$ on the one hand and between $Y_{(LPS\ +\ MV)}$ and $O_{(LPS\ +\ MV)}$ on the other hand.
Conclusion: Middle-old mice were more susceptible to both LPS alone and the combination of LPS and low tidal volume MV as compared to their young adult counterparts. The synergism between LPS and MV was amplified in middle-old mice.

1. Introduction

Acute respiratory distress syndrome (ARDS) is a life threatening condition consisting in an acute respiratory failure caused by a non-hydrostatic pulmonary edema responsible for hypoxemia and radiological bilateral lung infiltrates (Ranieri et al., 2012). Despite decades of medical research and major improvements in ARDS patient care, the mortality still remains high, around 40% (Bellani et al., 2016). A meta-analysis has recently identified age as the sole clinical independent predictive factor for mortality during ARDS (Phua et al., 2009). Some determinants of this "age-related" severity have been explored in studies using rodent models of ARDS with injurious mechanical ventilation (MV) (Nin et al., 2008; Setzer et al., 2013) and lipopolysaccharide (LPS) administration (Gomez et al., 2007). These studies suggested an enhanced lung inflammation and systemic vascular dysfunction in old animals (Gomez et al., 2007). However, these reports have several limitations: i) none has examined baseline age-associated changes in lung structure (Meyer,

Abbreviations: ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; ELISA, enzyme-linked immunosorbent assay; FOT, force oscillation technique; IL-6, interleukin-6; LPS, lipopolysaccharide; MIP-2, macrophage inflammatory protein-2; MV, mechanical ventilation; TNFα, tumor necrosis factor-α.

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http://dx.doi.org/10.1016/j.exger.2017.06.022
0531-5565/

2005) and lung mechanics; ii) none has explored pulmonary hemodynamics, although pulmonary vascular dysfunction has been shown to be associated with poor outcome during ARDS (Boissier et al., 2013; Bull et al., 2010; Mekontso Dessap et al., 2016); iii) none has used a clinically relevant model of ARDS.

We herein report on a double hit murine model of ARDS, combining LPS aspiration (as lung infection is the most frequent cause of ARDS in humans (Rubenfeld et al., 2005)) and a low tidal volume mechanical ventilation (MV) as commonly used in clinical practice (Esteban et al., 2008) applied to young adult (10–12 weeks) and middle-old (12–13 months) wild-type C57Bl6 mice. We investigated the determinants of the age-related severity of ARDS, with a focus on lung mechanics and pulmonary hemodynamics in addition to lung injury, inflammation and edema.

2. Materials and methods

2.1. Animals

Young adult and middle-old wild-type C57Bl6 male mice, respectively 11 ± 1.7 and 52 ± 0.5 weeks old, were used (Janvier, Le Genest Saint Isle, France). All animal care and procedures were performed after approval of the Institutional Animal Care Committee, in accordance with the official regulations of the French Ministry of Agriculture guidelines for the experimental use of animals.

2.2. Pilot experiments to adjust tidal volume per body weight

We harvested lungs from young adult ($n = 4$) and middle-old ($n = 4$) mice to measure the lung weight/body weight ratio, which was similar in both groups: 0.0057 ± 0.00020 *vs.* 0.0056 ± 0.00026, $p = 0.686$. We therefore decided to administer the tidal volume according to body weight as previously described in rats (Nin et al., 2008).

2.3. Animal protocol

Mice were anesthetized with inhaled 5% isoflurane (Abbott, Rungis France) and were kept in spontaneous breathing; 1.5 μl/g of body weight volume instillate consisting of 2 μg/μl of *Escherichia coli* 055:B5 derived lipopolysaccharide (Sigma-Aldrich Chimie, Lyon, France) or control saline was delivered into the distal part of the oropharynx and aspirated into the lower respiratory tract. After oropharyngeal aspiration, mice woke up and returned to their cage with free access to water and food. Twenty-four hours later (H24), mice were reanesthetized with intraperitoneal pentobarbital (30 μg/g of body weight, Hospira, Meudon La Forêt, France) followed by continuous 5% isoflurane (Abbott, Rungis France). Mice were either immediately sacrificed, or underwent intubation for MV (see Experimental design). For MV, the larynx was surgically exposed and the trachea was intubated orally under direct vision with a metal cannula (internal diameter of 1 mm, Harvard Apparatus, Les Ulis, France). The tracheal cannula was properly secured with surgical thread (Ethicon 3-0, Ethicon, Auneau, France) before connection to mechanical ventilator in order to avoid leaks. The cervicotomy was closed with surgical thread (Ethicon 6.0, Ethicon, Auneau, France).

2.4. Mechanical ventilation

Mice were ventilated in the supine position using humidified gas (20 mg H_2O/L absolute humidity, MR410 humidifier, Fischer & Paykel Healthcare, Courtaboeuf, France), with a tidal volume of 8 μL/g of body weight, a respiratory rate of 180/min, an end-expiratory pressure of 3 cm H_2O, and a fraction of inspired oxygen of 0.5, by means of a computer-driven small-animal ventilator (flexiVent, Scireq, Montreal, Canada). These ventilator parameters have been shown to provide suitable minute ventilation (Mekontso Dessap et al., 2012). Mechanical ventilation lasted 3 h with continuous anesthesia maintained by 1.5% isoflurane and muscle paralysis using intraperitoneal pancuronium at the onset of the experiment and every hour (0.8 μg/g of body weight, Organon, Puteaux, France) to ensure passive mechanical conditions. Mice received intraperitoneal warm fluid boluses (5% dextrose with 9 g/L NaCl) at the onset of the experiment (20 μL/g of body weight) and every hour (10 μL/g of body weight).

2.5. Experimental design

The experimental design included eight groups: young adult mice exposed to control saline aspiration [$Y_{(control)}$], middle-old mice exposed to control saline aspiration [$O_{(control)}$], young adult mice exposed to LPS aspiration [$Y_{(LPS)}$], middle-old mice exposed to LPS aspiration [$O_{(LPS)}$], young adult mice exposed to control saline aspiration and MV [$Y_{(MV)}$], middle-old mice exposed to control saline aspiration and MV [$O_{(MV)}$], young adult mice exposed to LPS aspiration and MV [$Y_{(LPS\ +\ MV)}$], and middle-old mice exposed to LPS aspiration and MV [$O_{(LPS\ +\ MV)}$]. Each group included 13 to 16 animals.

2.6. Lung mechanics

Special features of the *flexiVent* ventilator include a continuous monitoring of airway pressures and a precision computer-controlled piston that is capable of accurately measuring the delivered volume (with appropriate corrections for gas compression) and to produce any desired waveform, allowing respiratory mechanics assessment with the forced oscillation technique (FOT) and pressure-volume curves (Mekontso Dessap et al., 2012). Mice were allowed to stabilize on the ventilator for 5 min and were then inflated twice to a transrespiratory pressure of 30 cm H_2O to establish a standard volume history. Respiratory system dynamic compliance and elastance (C_{dyn} and E_{dyn}) were measured using the single frequency FOT at initiation of MV, before and after volume history standardization, and then hourly to capture the time course and the detailed response to MV. The respiratory system quasi-static compliance (C_{qst}) was measured using a pressure-driven pressure-volume curve at start (before and after volume history standardization) and at end of MV. Before the end of the timed ventilator protocol, mice underwent hemodynamic explorations (Mekontso Dessap et al., 2012).

2.7. Hemodynamic explorations

Through the cervical midline incision, both the right jugular vein and left carotid artery were isolated using a stereomicroscope (Leica MZ 7.5, Leica Microsystems, Nanterre, France). An ultra-miniature 0.47 mm high fidelity pressure transducer catheter (SPR-671, Millar Instruments, Houston, TX) was inserted into the right jugular vein and advanced into the right ventricle. The micromanometer was calibrated *in vitro*, firstly electronically and secondly against a column of mercury with the reference zero level taken at mid chest. Right ventricular systolic pressure was measured during a short end-expiratory ventilatory pause using a Gould transducer (Gould, Cleveland, USA) and a Notocord system (Emka Technologies, Paris, France). The pressure transducer catheter was then inserted into the left carotid artery to measure systemic blood pressure and cardiac rate. Cardiac output was measured by the transpulmonary thermodilution technique (Champion et al., 2000). Briefly, a 0.34 mm external diameter thermistor microprobe (Columbus Instruments, Columbus, OH) was inserted into the left carotid artery and advanced to the aortic arch, where changes in aortic blood temperature were measured. A 0.5 mm external diameter catheter placed in the right jugular vein was advanced to the right atrium for bolus injection of 20 μL of NaCl 9 g/L solution at 20 °C. Five consecutive cardiac output measurements were obtained using the Cardiomax-III system (Columbus Instruments) and total pulmonary vascular resistances