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Bronchial responsiveness in an elastase-induced mouse model of emphysema

Luiz Marcelo Oliveira Santos[a], Daniela Aparecida de Brito Cervilha[a], Layla Dutra Marinho Cabral[a], Érika Kristina Incerpi Garcia[a], Vanessa Pereira Teixeira[a], Jôse Mára Brito[b], Henrique Takachi Moriya[c], Roseli Soncini[a,d,*]

[a] Laboratory of Physiology, Federal University of Alfenas, Minas Gerais, Brazil
[b] Laboratory of Lung Pathology, University of São Paulo, São Paulo, Brazil
[c] Department of Biomedical Engineering, University of Sao Paulo, São Paulo, Brazil
[d] Department of Biomedical Engineering, Boston University, Boston, MA, USA

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ABSTRACT

Bronchial responsiveness during methacholine (MCh) challenge was analysed in an elastase-induced mouse model of emphysema to explore the magnitude of the response in this model. Swiss mice were intratracheally instilled with saline or elastase (0.3 or 0.6 U). Twenty days afterward, mechanical ventilation data were collected from the closed and opened thorax of baseline and MCh (vehicle, 50 and 100 mg/mL) challenged mice. The lungs were prepared for morphometric analysis. In the 0.6 U group, airway resistance (R_{aw}) and tissue elastance (H) were decreased, and hysteresivity (η) was increased (closed thorax). MCh increased R_{aw}, G and H in all groups, but this increase was attenuated in the elastase-induced emphysema groups, the largest attenuation was observed in the 0.6 U (closed thorax condition). Elastase increased hyperinflation of the alveoli, alveolar collapse and the Lm and reduced the normal area. MCh reduced respiratory mechanics in elastase-induced emphysema, and this reduction was modulated by the collapsed and/or hyperinflated areas, which increased the heterogeneity of the lungs.

1. Introduction

Emphysema is a type of chronic obstructive pulmonary disease (COPD) and is a worldwide public health problem that reduces quality of life (Hogg and Timens, 2009). Emphysema is characterised by airspace enlargements and is accompanied by destruction of the parenchymal structure (Snider, 1989). The causes of emphysema in humans are cigarette smoke, environmental irritants, genetic factors and indoor pollutants (Barnes, 2000); these factors result in general decrements in health that include weight loss, muscle atrophy, changes in muscle fibre type and systemic inflammation (Langen et al., 2006). Emphysema leads to death, and there is currently no cure. The emphysema observed in smokers begins in the respiratory bronchioles near the thickened and narrowed small bronchioles that become the major site of obstruction in COPD (Hogg and Timens, 2009). The physiological manifestations of the disease include impaired gas exchange (due to loss of alveolar surface area), limited airflow, increased lung compliance and increased effort required to breathe (Hogg, 2004).

Because cigarette smoke is the main cause of emphysema in humans, experimental animal models have attempted to reproduce this situation (Churg et al., 2008). Elastolytic enzymes have been shown to reproduce some characteristics of human cigarette smoke-induced disease (Breuer et al., 1993; Ito et al., 2005).

Methacholine (MCh) is a bronchoconstrictive agent that has been widely used in the diagnoses of airway narrowing and hyperresponsiveness (Jonasson et al., 2009). MCh induces muscle contractions by stimulating the muscarinic cholinergic receptors that are found in both the airways and the lung parenchyma (Barnes, 1993; Sly et al., 1995; Fisher et al., 2004). Muscarinic receptors located on the alveolar wall may be involved in the parenchymal response (Sly et al., 1995).

The hypothesis of this study is that bronchial responsiveness during aerosolised MCh challenge should be amplified in mice with elastase-induced emphysema (0.3 and 0.6 U elastase). This study also compared two thorax conditions: intact (closed thorax) and exposed lungs (opened thorax). The aim of this study was to analyse

* Corresponding author at: Institute of Biomedical Sciences, Federal University of Alfenas-MG, Rua Gabriel Monteiro da Silva, 700, 37130-000 Alfenas, MG, Brazil. Tel.: +55 35 3299 1405; fax: +55 35 3299 1063.
E-mail address: soncinir@yahoo.com.br (R. Soncini).

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the bronchial responsiveness of the airways to a MCh challenge during elastase-induced emphysema in closed and opened thorax.

2. Materials and methods

2.1. Animals

Forty-eight male Swiss mice (25–30 g) were maintained on a 12 h light cycle and fed with standard chow and water *ad libitum* in the animal care facility of the Laboratory of Physiology, Federal University of Alfenas. The experiments were conducted in accordance with the Declaration of Helsinki for the welfare of experimental animals, and the Ethics Committee of the Federal University of Alfenas approved the experimental methods (protocol number 472/2012).

2.2. Animal preparation

The mice were anesthetised using ketamine (34 mg/kg, intraperitoneal, *i.p.*) and xylazine (12 mg/kg, *i.p.*), an anterior cervical incision exposed the trachea, and one of the following solutions was instilled: sterile saline solution (0.9% NaCl, 50 μL; Sal group) or porcine pancreatic elastase (PPE; 0.3 or 0.6 U, Sigma, St. Louis, MO, USA) diluted in 50 μL of saline solution (elastase-induced emphysema groups). Two PPE doses were used to analyse structural changes in the lungs and the consequences to airway responsiveness.

2.3. Respiratory mechanics

Twenty days after instillation, respiratory mechanics were assessed. Each animal was anesthetised (pentobarbital sodium, 68 mg/kg, *i.p.* and xylazine, 12 mg/kg, *i.p.*), tracheostomised (18-gauge metal IV adaptor), and mechanically ventilated with a tidal volume of 10 mL/kg, a breathing frequency of 120 breaths/min, and 3 cm of H_2O positive end expiratory pressure (PEEP) using a small animal ventilator (flexiVent, SCIREQ, Montreal, Quebec, Canada). The animals were paralysed with an injection of pancuronium bromide (0.5 mL/kg, *i.p.*) and kept warm using a heated nest. The respiratory system input impedance (Zrs) was measured by applying 3 s of oscillatory volume perturbation to the tracheal cannula that was connected to the airway opening. By fitting the constant phase model (Hantos et al., 1992) to the obtained data, the mechanical parameters of airway resistance (R_{aw}), tissue damping (G), tissue elastance (H) and hysteresivity (η) were estimated. This technique was specially designed to measure the input Zrs of small animals (Gomes et al., 2000) and has been described in detail previously (Hantos et al., 1992; Peták et al., 1997).

The experiments were conducted with the chest wall preserved (closed thorax) and, in a different group, with the lungs exposed to minimise the mechanical effects of the chest wall (opened thorax). The thoracotomy was performed on anesthetised mice under mechanical ventilation and was completed within 15 min. According to the approved protocol, the levels of anaesthesia were measured *via* pain withdrawal thresholds to pressure and, if necessary, a complementary dose was administered.

The bronchial responsiveness of each mouse was tested. A saline solution (vehicle) and methacholine concentrations of 50 and 100 mg/mL (MCh, acetyl-β-methylcholine chloride, St. Louis, USA) were aerosolised using an ultrasonic device over a period of 10 s (Aeroneb, Aerogen, Ireland). After 45 s, respiratory mechanics were assessed over the next 3 min. The time intervals between the deliveries of each solution were 5 min. To standardise lung volume histories, the lungs were inflated twice to a pressure of 30 cmH_2O (recruitment manoeuvre) before the initiation of solution delivery. For each mouse, the parameters of respiratory mechanics were obtained from the correct values of coefficient of determination (*i.e.*, a control parameter measuring the fitness of the model) and were plotted against MCh concentration (0, 50 and 100 mg/mL). The animals were euthanised by rapid exsanguination *via* the abdominal aorta while anesthetised.

2.4. Morphometry

After mechanical ventilation, the anterior chest wall was surgically removed and a positive end-expiratory pressure of 3 cmH_2O applied. A laparotomy was performed and heparin (1000 IU) was intra-venously injected into the vena cava. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive haemorrhage that quickly killed the animals (Cruz et al., 2012). The right lung was removed, fixed in 10% buffered paraformaldehyde and embedded in paraffin. Four-micron thick slides were cut and stained with haematoxylin–eosin. The morphology of the lung architecture was analysed with an integrating eyepiece on a coherent system made up of a 100-point grid consisting of 50 lines of known length and coupled to a conventional light microscope (Nikon, Japan). The volume fractions of the collapsed, hyperinflated and normal pulmonary areas were determined using the point-counting technique (Weibel, 1990) at a magnification of 200×. The air spaces were evaluated using the mean linear intercept (Lm) measurement, in which twenty random areas in non-coincident microscopic fields were chosen, and the numbers of the alveoli-line intercepts were counted (Dunnill, 1964).

2.5. Statistical analyses

The results are expressed as the means ± the standard errors of mean (SEM). Statistical significance was assessed with parametric methods; two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test were used to analyse different conditions (saline, 0.3 and 0.6 U) and methacholine concentrations (0, 50 and 100 mg/mL). For each analysis, $p < 0.05$ was considered statistically significant. The statistical analysis and graphs were performed and created with GraphPad Prism (version 6.0, San Diego, CA, USA).

3. Results

The baseline respiratory mechanical data are presented in Table 1. The open or closed thorax conditions and the Sal, 0.3 and 0.6 U groups were compared with two-way analysis of variance. The elastase-induce emphysema significantly decreased R_{aw} (closed thorax), and the expected H decrease was found in the 0.6 U group ($p < 0.05$; closed and opened thorax). Additionally, η increased for the same group (closed thorax) relative to that of the Sal group. No changes in G were found.

The MCh challenge is illustrated in Fig. 1, and all groups (in both the closed and opened thorax conditions) showed dose-dependent responses to aerosolised MCh (0 or vehicle, 50 and/or 100 mg/mL) for all parameters except H in the 0.6 U group (closed thorax condition), for which only 100 mg/mL MCh resulted in a statistically significant difference relative to the H for the vehicle.

The responses to 50 mg/mL MCh in the control and elastase-induced emphysema groups did not significantly differ. However, the R_{aw} response to 100 mg/mL MCh in the 0.6 U elastase-induced emphysema group (0.80 ± 0.07 cmH_2O s/mL) was reduced when compared to that of the control group (1.51 ± 0.33 cmH_2O s/mL; $p < 0.005$) in the closed thorax condition, but no significant response was found in the opened thorax condition. G was significantly reduced in the 0.6 U elastase-induced emphysema group (9.26 ± 0.80 cmH_2O/mL) relative to that of the control group