Respiratory Physiology & Neurobiology 200 (2014) 90–96

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Papain-induced experimental pulmonary emphysema in male and female mice

Mariana Nascimento Machado[a], Silviane Fernandes da Silva Figueirôa[a], Flavia Mazzoli-Rocha[a], Samuel dos Santos Valença[b], Walter Araújo Zin[a,*]

[a] Laboratory of Respiration Physiology, Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Laboratory of Integrative Histology, Institute of Biomedical Sciences, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Accepted 6 June 2014
Available online 12 June 2014

Keywords:
Pulmonary emphysema
Papain
Lung mechanics
Elastance
Histology
Oxidative stress

ABSTRACT

In papain-induced models of emphysema, despite the existing extensive description of the cellular and molecular aspects therein involved, sexual hormones may play a complex and still not fully understood role. Hence, we aimed at exploring the putative gender-related differences in lung mechanics, histology and oxidative stress in papain-exposed mice. Thirty adult BALB/c mice received intratracheally either saline (50 μL) or papain (10 U/50 μL saline) once a week for 2 weeks. In males papain increased lung resistive and viscoelastic/inhomogeneous pressures, static elastance, and viscoelastic component of elastance, while females showed higher static elastance and resistive pressure only. Both genders presented similar higher parenchymal cellularity and mean alveolar diameter, and less collagen-elastic fiber content and body weight gain than their respective controls. Increased functional residual capacity was more prominent in males. Female papain-treated mice were more susceptible to oxidative stress. Thus, male and female papain-exposed mice respond differently, which should be carefully considered to avoid confounding results.

1. Introduction

The literature suggests that gender is an important factor in determining risk and prognosis in many diseases, including emphysema. However, the reports are contradictory. According to Martinez et al. (2007) patients with severe COPD, women, relative to men, exhibit anatomically smaller airway lumens with disproportionately thicker airway walls and emphysema that is less extensive and characterized by smaller hole size and less peripheral involvement. It has also been described that women present less severe emphysema at all stages of COPD than man (Dransfield et al., 2007; Sverzellati et al., 2009).

Female sex hormones, particularly estrogen, significantly influence normal airway function as well as respiratory disorders, such as asthma (Ticconi et al., 2013). These actions are very complex and broad, directly on airway reactivity or indirectly through regulation of the immune and inflammatory responses in the lung (Ticconi et al., 2013). There is now a consensus that inflammation is at the heart of the pathobiology of COPD and that the inflammatory process involves both the lung (airways and parenchyma) and the systemic circulation (Barnes et al., 2003). In this line, the literature reports that emphysematous women present a higher degree of inflammation and bronchial hyperreactivity than men (O'Byrne et al., 1984; Barnes et al., 2003).

In a well-characterized cohort of patients, no difference in mortality was noted between males and females with severe emphysema (Martinez et al., 2007). In contrast, the survival rate of women was significantly better than that of men, i.e., female patients with COPD have a better prognosis than males (Miyamoto et al., 1995). However, females and males with the same level of obstructive lung disease appear to have the same level of mortality. Nevertheless, using standardized mortality rates, females have a higher mortality than males, suggesting that the protective effect of being female is lost in COPD patients (Ringbaek et al., 2005).

Mice models of lung diseases report that male sexual hormones exacerbate lung function impairment after bleomycin-induced fibrosis (Voltz et al., 2008), while estrogen is required for the

* Corresponding author at: Laboratório de Fisiologia da Respiração, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av Carlos Chagas Filho 373, CCS, Rm G2-042, Ilha do Fundão, 21941-902, Rio de Janeiro, RJ, Brazil. Tel.: +55 21 3938 6557; fax: +55 21 2280 8193.
E-mail addresses: wazin@biof.ufrj.br, walter_zin@hotmail.com (W.A. Zin).

http://dx.doi.org/10.1016/j.resp.2014.06.005
1569-9048/

maintenance of alveoli (Massaro and Massaro, 2004), prevents airway dysfunction (Dimitropoulou et al., 2009), modulates immune responses (Hsieh et al., 2007; Simoes et al., 2012), and protects the lung following trauma-hemorrhage (Hsieh et al., 2007). Additionally, the activation of estrogen receptors in the mitochondria is known to trigger anti-inflammatory signals, affecting reactive oxygen species and induction of apoptosis (Simoes et al., 2012).

Given the complexity of the pathophysiology of pulmonary emphysema and that the effects of sexual hormones on lung physiology and pathophysiology are still highly complex and not completely understood, we, thus, aimed at disclosing the putative gender-dependent mechanical, histological and biochemical lung impairment in a murine model of papain-induced emphysema.

2. Methods

2.1. Animal preparation

To carry out tracheal instillation, the animals were sedated by inhalation of sevoflurane, weighed (Filizola, model BR, Filizola Industries SA, SP, Brazil), and an ostium was created in the anterior trachea to allow the administration of saline or papain by means of an insulin syringe.

Thirty BALB/c female and male mice (20–25 g) were randomly divided into 4 groups. In SALF (female, $n = 6$) and SALM (male, $n = 6$) groups, mice were intratracheally (i.t.) injected with 0.05 mL of sterile saline solution (SAL) (0.9% NaCl) once a week for two weeks. In PAPF (female, $n = 9$) and PAPM (male, $n = 9$) groups, mice were i.t. injected with 0.05 mL of sterile saline solution (0.9% NaCl) containing 10 IU of papain (PAP) at a concentration of 0.2 IU/μL once a week for two weeks. Papain (USP 225310, Becton Dickinson Co., Franklin Lakes, NJ, USA) had been previously activated in 0.1 M sodium phosphate buffer containing 10 mM EDTA, 0.4 NaCl and 5 mM DTT (dithiothreitol) for 10 min at 40 °C. All animals were studied 21 days after the first saline or papain administration.

2.2. Pulmonary mechanics

On the 21st day after the first instillation, the animals were sedated with diazepam (1 mg *i.p.*) and anesthetized with pentobarbital sodium (20 mg/kg body weight *i.p.*), paralyzed with pancuronium bromide (0.1 mg/kg body weight *i.v.*), and mechanically ventilated with air (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) with a frequency of 100 breaths/min, tidal volume of 0.2 mL, inspiratory flow of 1 mL/s, and positive end-expiratory pressure (PEEP) of 2 cmH_2O. The anterior chest wall was surgically removed.

Lung mechanics was determined as previously described (Machado et al., 2014). Briefly, we determined lung resistive ($\Delta P1$) and viscoelastic/inhomogeneous ($\Delta P2$) pressures, static elastance (Est), and viscoelastic component of elastance (ΔE) by the end-inflation occlusion method (Bates et al., 1985). $\Delta P1$ selectively reflects airway resistance, and $\Delta P2$ represents stress relaxation or viscoelastic properties and mechanical heterogeneities of the lung (Bates et al., 1989; Saldiva et al., 1992). Lung mechanics was measured 10–15 times in each animal.

2.3. Histological study

Heparin (1000 IU) was injected into the abdominal vena cava right after the determination of respiratory mechanics. The trachea was clamped at end expiration, remaining the lung at functional residual capacity. The abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly euthanized the animals. The lungs were removed. The right one was frozen in liquid N_2 for posterior biochemical analyses, whereas the left lung was submerged in buffered 10% formaldehyde for twenty-four hours (Millonig's phosphate buffer: 100 mL HCHO, 900 mL H_2O, 18.6 g NaH_2PO_4, 4.2 g NaOH). After fixation, the tissue was embedded in paraffin. Four-μm-thick slices were obtained, mounted on glass slides, and stained with either hematoxylin–eosin (for the determination of lung cellularity and alveolar diameter), or Weigert's Resorcin-Fuchsin modified with oxidation (elastic fibers) or picrosirius (collagen fibers).

Lung slides were analyzed by optical microscopy (Axioplan microscope, Zeiss, Oberkochen, Germany). Quantitative analysis was performed by the point-counting technique (Weibel, 1990) across 10 random non-overlapping microscopic fields. The number of mononuclear (MN) and polymorphonuclear (PMN) cells in the pulmonary tissue was evaluated at 1000× magnification. Points falling on MN and PMN cells were counted, and divided by the total number of points falling on tissue area in each microscopic field (Gundersen et al., 1988; Weibel, 1990). For the determination of mean alveolar diameter (Lm) the number of alveolar intercepts in 20 random fields in each sample were counted at 200× magnification, and Lm was calculated as the sum of line segments (1250 μM each)/number of intercepts.

The area of lung parenchyma occupied by collagen and elastic fibers was identified by the point-counting technique on images captured in a blinded manner across 10 random non-coincident fields (400× magnification). The quantification was done on captured high quality images (2048 × 1536 pixels) using the Image Pro Plus 4.5.1 software (Media Cybernetics, Silver Spring, MD, USA). Results were expressed as percentage of points falling on fibers divided by the number of points hitting lung tissue.

Functional residual capacity (FRC) was determined by the volume displacement technique (Scherle, 1970).

2.4. Biochemical assays

Biochemical assays were done in right lung homogenates. Activity of the antioxidant enzyme catalase (CAT) was measured by the rate of decrease of hydrogen peroxide concentrations monitored at 240 nm (Aebi, 1984). CAT values were corrected for the amount of protein in each sample (U catalase/mg protein).

The glutathione/glutathione disulfide (GSH/GSSG) ratio was estimated in lung homogenates by first reacting GSH and GSSG with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and measuring the 2-nitro-5-thiobenzoate (TNB) chromophore produced in the reaction. To determine the concentration of GSSG, samples were treated with 2-vinylpyridine, which covalently reacts with GSH (but not with GSSG). The excess 2-vinylpyridine was neutralized with triethanolamine. The rate of formation of TNB, measured at 412 nm, is proportional to the concentration of GSSG in the sample. GSH or GSSG concentrations in the samples were determined from standard curves generated with different concentrations of purified GSH or GSSG (Rahman et al., 2006).

As an index of oxidative damage induced by lipid peroxidation, we used the thiobarbituric acid reactive substances (TBARS) method to analyze malondialdehyde (MDA) products during an acid-heating reaction, as previously described by Draper et al. (1993). Briefly, samples from lung homogenate were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid; the samples were then heated in a boiling water bath for 30 min. TBARS levels were determined by absorbance at 532 nm. The MDA values were normalized by the protein content in the samples (nmol/mg protein).

The total protein content in the samples from lung homogenates was determined by Bradford's method (1976).