Respiratory Physiology & Neurobiology 195 (2014) 27–36

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Effects of early and late pneumothorax drainage on the development of pulmonary oedema

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ARTICLE INFO

Article history:
Accepted 11 February 2014

Keywords:
Alveolar cell biology
Alveolar-capillary permeability
Inflammation
Lung oedema
Alveolar fluid clearance

ABSTRACT

We analyzed the effects of pneumothorax duration and early or late drainage on lung histology and biological markers associated with inflammation, alveolar fluid clearance, and pulmonary oedema formation. Pneumothorax was induced by injecting air into the thorax of anaesthetized rats, which were randomized according to duration of pneumothorax [5 (PTX5) or 30 (PTX30) min] and further divided to be drained (D) or not (ND). ND rats were euthanized at 5 and 30 min. In D groups, pneumothorax was drained and rats breathed spontaneously for 30 min. PTX30-ND, compared to PTX5-ND, showed higher alveolar collapse and oedema, type III procollagen, caspase-3, epithelial sodium channel-α, and aquaporin (AQP)-1 mRNA expression, and epithelial and endothelial damage, with reduced cystic fibrosis transmembrane conductance regulator (CFTR) and AQP-3 expression. PTX5-D, compared to PTX30-D, showed less alveolar hyperinflation, oedema, and alveolar-capillary damage, with reduced interleukin-6, caspase-3, AQP-5, and Na,K-ATPase-α and -β expression, and increased CFTR expression. In conclusion, longer duration pneumothorax exacerbated lung damage, oedema, and inflammation.

1. Introduction

Pneumothorax is a pathological condition in which air accumulates within the pleural cavity as a result of trauma or underlying disease (Noppen, 2010). Pneumothorax of long duration may lead to increased alveolar collapse and interstitial oedema (Maranhao et al., 2000). Moreover, pulmonary oedema may be related to re-inflation of the collapsed lung, an event known as "re-expansion pulmonary oedema" (REPO) (Neustein, 2007). Mortality occurs in up to 20% of REPO cases, probably caused by an abrupt reduction in pleural pressure as a result of extensive pneumothorax drainage or long-term pulmonary collapse (Echevarria et al., 2008). REPO has been associated with the release of pro-inflammatory mediators, neutrophil infiltration (Nakamura et al., 2000; Sakao et al., 2001; Funakoshi et al., 2004), and decreased surfactant levels (Sewell et al., 1978). A common end-point seems to be increased permeability sometimes associated with disruption of the alveolar-capillary membrane and ischaemia/reperfusion-mediated injury (Sivrikoz et al., 2002).

Different mechanisms regulate pulmonary oedema formation and resolution. Pulmonary oedema may result either from increased driving pressure for fluid infiltration (cardiogenic oedema) or from a weakening of epithelial and endothelial barriers that normally restrain fluid flow and protein movement (increased permeability, non-cardiogenic oedema) (Murray, 2011). In turn, oedema fluid clearance is accomplished by active ion transport. Sodium transport across the alveolar epithelium is regulated by

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http://dx.doi.org/10.1016/j.resp.2014.02.004
1569-9048/

apical Na^+ (Voilley et al., 1994; Yue et al., 1995) and chloride channels (O'Grady et al., 2000; Jiang et al., 2001), and by basolateral Na,K-ATPase (Ridge et al., 1997). An osmotic gradient is established making the water flow passively, in part through water channels called aquaporin (AQP), from the air spaces into the interstitium and pulmonary circulation (Song et al., 2000). To the best of our knowledge, however, no study has evaluated the role of these channels in oedema formation during pneumothorax.

The aim of this study was to analyze the effects of pneumothorax duration and early or late drainage on lung histology and biological markers associated with inflammation [interleukin (IL)-6 and IL-1β], apoptosis (caspase-3), fibrogenesis [type III pro-collagen (PCIII)], and alveolar fluid clearance and pulmonary oedema formation [epithelial sodium channel (ENaC)-α, cystic fibrosis transmembrane conductance regulator (CFTR), Na,K-ATPase (α and β subunits), and AQP-1, AQP-3, and AQP-5] in anaesthetized, spontaneously breathing rats.

2. Materials and methods

This study was approved by the Ethics Committee of the Health Sciences Centre, Federal University of Rio de Janeiro, Brazil. 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 National Academy of Sciences, USA.

2.1. Animal preparation and experimental protocol

Thirty adult male Wistar rats (350–380 g) were used. All rats were sedated (diazepam, 5 mg intraperitoneally), anaesthetized (thiopental sodium, 20 mg/kg intraperitoneally), and tracheotomised. Rats were then randomly divided into two groups: control (C, $n = 6$) or pneumothorax (PTX, $n = 24$). Pneumothorax was induced by injecting 8 mL of air into the right hemi-thorax, and adequacy was confirmed radiographically (Fig. 1). PTX animals were further randomized according to duration of pneumothorax: 5 min (PTX5, $n = 12$) or 30 min (PTX30, $n = 12$). Both PTX groups were again subdivided to be drained (D, $n = 6$) or not drained (ND, $n = 6$). ND animals were euthanized at 5 and 30 min. In D groups, pneumothorax was drained at 5 min (early drainage, PTX5-D) or 30 min (late drainage, PTX30-D) and rats were then allowed to breathe spontaneously for 30 min (Fig. 2). C rats were anesthetized and breathed spontaneously for 5 or 30 min before euthanasia. At each time point, a laparotomy was performed, heparin (1000 IU) was injected intravenously in the vena cava. Sodium thiopental (50 mg/mL) was injected to increase the depth of anaesthesia, after which 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.

2.2. Light microscopy

The right lung was quick-frozen by immersion in liquid nitrogen, fixed in Carnoy's solution, and embedded in paraffin. Four-μm-thick slices were cut and stained with haematoxylin–eosin. Lung morphometric analysis was performed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines (known length) coupled to a conventional light microscope (Olympus BX51, Olympus Latin America-Inc., Brazil). Two investigators who were unaware of the origin of the material examined the samples microscopically. The slides were coded and examined only at the end of all measurements. The volume fraction 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), normal lung areas (those not showing overdistended or plicate walls), and alveolar oedema was determined by the point-counting technique (Weibel, 1990) at a magnification of 200× across 10 random, non-coincident microscopic fields. Briefly, points falling on hyperinflated or collapsed alveoli or on normal lung areas or alveoli with oedema were counted and divided by the total number of points in each microscopic field.

To quantify interstitial oedema, five arteries were transversely sectioned. The number of points falling on areas of perivascular oedema (NP) and the number of intercepts between the lines of the integrating eyepiece and the basement membrane of blood vessels (NI) were counted. The interstitial perivascular oedema index was calculated as follows: $NP^{1/2}/NI$ (Santiago et al., 2010).

2.3. Transmission electron microscopy (TEM)

Three slices measuring 2 mm × 2 mm × 2 mm were cut from three different segments of the right lung and fixed [2.5% glutaraldehyde and 0.1 M phosphate buffer (pH = 7.4)] for TEM (JEOL 1010 transmission electron microscope, Tokyo, Japan). For each TEM image (20/animal), the following components of structural damage were analyzed: (a) type I pneumocytes, (b) type II pneumocytes, (c) endothelial cells, (d) basement membrane, and (e) alveolar and interstitial oedema. Pathologic findings were graded according to a 5-point semi-quantitative severity-based scoring system as: 0 = normal lung parenchyma, 1 = changes in 1–25%, 2 = changes in 26–50%, 3 = changes in 51–75%, and 4 = changes in 76–100% of the examined tissue (Silva et al., 2010). All histological data were analyzed in a blinded fashion by two pathologists.

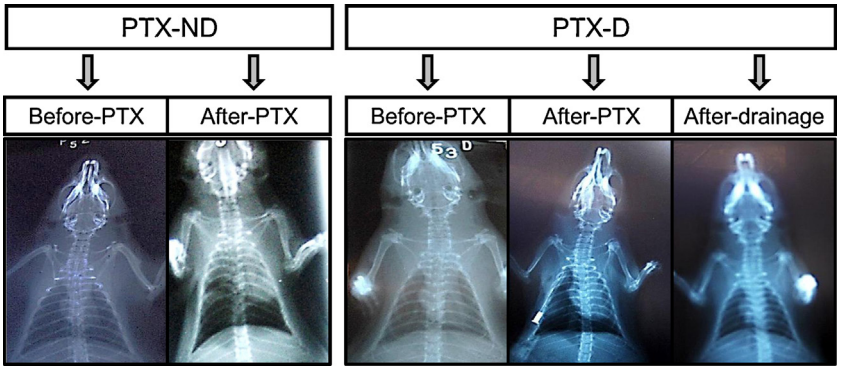

Fig. 1. Chest X-ray in a representative rat before and after pneumothorax (PTX-ND) as well as before, after PTX, and after drainage (PTX-D).