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Review

Lung and brainstem cytokine levels are associated with breathing pattern changes in a rodent model of acute lung injury☆

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ABSTRACT

Acute lung injury evokes a pulmonary inflammatory response and changes in the breathing pattern. The inflammatory response has a centrally mediated component which depends on the vagi. We hypothesize that the central inflammatory response, complimentary to the pulmonary inflammatory response, is expressed in the nuclei tractus solitarii (nTS) and that the expression of cytokines in the nTS is associated with breathing pattern changes. Adult, male Sprague-Dawley rats ($n = 12$) received intratracheal instillation of either bleomycin (3 units in 120 μl of saline) or saline (120 μl). Respiratory pattern changed by 24 h. At 48 h, bronchoalveolar lavage fluid and lung tissue had increased IL-1β and TNF-α levels, but not IL-6. No changes in these cytokines were noted in serum. Immunocytochemical analysis of the brainstem indicated increased expression of IL-1β in the nTS commissural subnucleus that was localized to neurons. We conclude that breathing pattern changes in acute lung injury were associated with increased levels of IL-1β in brainstem areas which integrate cardio-respiratory sensory input.

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1. Introduction

Acute lung injury (ALI) is a common clinical problem characterized by diffuse, heterogeneous lung damage that is caused by both direct injury to the lung (e.g., pneumonia) and indirect insults (e.g., sepsis) (Ashbaugh et al., 1967; Bernard et al., 1994; Rubenfeld et al., 2005). Whether ALI results from a stimulus of local inflammation or as part of a systemic inflammatory process, insult to the lung disrupts the alveolar–capillary interface and initiates an inflammatory cascade (Ware and Matthay, 2000; Suratt and Parsons, 2006). This inflammatory response includes release of proinflammatory mediators, upregulation of adhesion molecules, recruitment of neutrophils and activation of lung macrophages with resultant production of early response cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Strieter et al., 1999; Ware and Matthay, 2000; Shimabukuro et al., 2003). If the lung is unable to recover from the initial insult or contain the inflammatory response, injury worsens progressing to the acute respiratory distress syndrome (ARDS) (Ware and Matthay, 2000; Piantadosi and Schwartz, 2004).

Breathing patterns are altered by the onset and progression of respiratory diseases (Kuratomi et al., 1985; Brack et al., 2002; Miyata et al., 2002, 2004; Yeragani et al., 2002; Bien et al., 2004; Giraldo et al., 2004; Casaseca-de-la-Higuera et al., 2006; Wysocki et al., 2006; Ibrahim et al., 2008; Veiga et al., 2010). The mechanisms responsible for changes in the breathing pattern in ALI and ARDS are attributed to the fundamental pathophysiology of diffuse pulmonary infiltrates, altered ventilation–perfusion matching, progressive hypoxemia, reduced lung compliance and increased work of breathing (Gattinoni et al., 1994; Pelosi et al., 1995). However, the relative contribution of each of these mechanisms has not been defined, and the impact of inflammation to altered respiratory pattern is unknown. Furthermore, while there is a growing appreciation of the importance of variability of biological signals (*i.e.*, variability in the pattern) (Kleiger et al., 1987; Lishner et al., 1987; Casolo et al., 1989; Odemuyiwa et al., 1991; Huikuri et al., 2000; Mietus et al., 2000; Goldberger, 2001; Griffin and Moorman, 2001; Tapanainen et al., 2002; Griffin et al., 2004; Stein et al., 2005), changes in breathing pattern variability in ALI and ARDS have not been characterized.

Most probably, multiple mechanisms contribute to changes in breathing pattern variability in ALI including alterations in lung mechanics and sensory feedback. For example, pathophysiological changes in the plant (lungs and muscles) will influence

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the temporal pattern of breathing, the variability from breath-to-breath. Reductions in vital capacity (VC) in the setting of gas exchange requirements that necessitate maintenance of a relatively normal tidal volume (V_T) may narrow the VC/V_T ratio and result in a more uniform rate and depth of pattern (Brack et al., 2002). Hypoxia (Jubran and Tobin, 2000; Van den Aardweg and Karemaker, 2002) and hypercapnia (Jubran et al., 1997; Fiamma et al., 2007), which result from lung injury, also alter variability of ventilatory patterns. Further, carotid body chemoreceptors are sensitized during early stages of lung injury (Jacono et al., 2006) and increased gain of the 'sensor' could act to increase variability of the controlled system (Cherniack et al., 1966; Longobardo et al., 1966). In chronic lung injury where the lung is fibrotic, vagal afferents drive the characteristic tachypnea (Schelegle et al., 2001). Thus, changes in magnitude and type of sensory input not only influence the breathing pattern but also the variance of the breathing pattern during illness, specifically acting to modulate breathing pattern variability in ALI (Bruce, 1996; Benchetrit, 2000; Webber and Zbilut, 2006).

However, afferent feedback and mechanical properties of the lung are not the only determinants of breathing patterns (Bruce, 1996; Benchetrit, 2000; Webber and Zbilut, 2006). Neural control of breathing can also influence pattern, and changes in breathing pattern variability may be adaptive. For example, a breathing pattern with low variability minimizes dyspnea in patients with restrictive lung disease (Brack et al., 2002); respiratory complexity decreases during slow-wave sleep (Sako et al., 2001) whereas it increases in patients with panic disorder (Yeragani et al., 2002), and complex waxing and waning respiratory patterns have been reported in an animal model of stroke with normal lung physiology (Koo et al., 2010). Thus, changes related to intrinsic properties of the nervous system such as state are mediated by the brainstem and contribute to changes in deterministic structure, variability and pattern of respiration (Dhingra et al., 2011).

Finally, local lung inflammation may influence breathing pattern variability. Endotoxin (Lai et al., 2005) and reactive oxygen species (Ruan et al., 2005) activate lung C fibers, which alter breathing frequency. Further, studies in humans identified a decrease in variability of respiratory timing with endotoxemia supporting an impact of systemic inflammation on breath-to-breath dynamics (Preas et al., 2001). However, the impact of a neural inflammatory response on breathing pattern variability in ALI is unknown. We hypothesize that in ALI, the mechanisms responsible for changes in variability of the respiratory pattern after lung injury relate to the central expression of cytokines and their influence on state. To begin to assess the role of local brainstem inflammation to breathing pattern responses in lung injury, we determined the association of lung and brainstem cytokine levels with changes in the linear and nonlinear properties of breathing pattern variability in the first 48 h of bleomycin-induced ALI.

2. Materials and methods

2.1. Experimental procedures

Male Sprague-Dawley rats (100–250 g, $n = 12$, Harlan) were purchased with their jugular vein catheterized and with the catheter exteriorized between their shoulder blades. Briefly, plethysmographic recordings were made at baseline (approximately 24 h after shipment) and then 6, 12, 24 and 48 h after intratracheal instillation of either saline (control group) or bleomycin. Venous blood samples (0.15 ml) were drawn at each of these time points. At 48 h the rats were exsanguinated and the following were collected: (a) bronchoalveolar lavage fluid (BALF), (b) lung tissue and (c) brainstem. The Institutional Animal Care and Use Committee of Case Western Reserve University approved the experimental protocols.

2.2. Chemically induced acute lung injury

We anesthetized the rats with a mixture of acepromazine, ketamine and xylazine using a weight-based dosing protocol. Once the rats were at a general surgical plane (no corneal reflex and no gag reflex) of anesthesia, the trachea was exposed by a 1-cm anterior neck incision. We inserted a 26-gauge needle between the cartilaginous rings of the trachea and administered bleomycin [3.0 units in 120 μl phosphate-buffered saline (PBS)] or 120 μl of PBS (control) (Jacono et al., 2006). The surgical site was closed with surgical tissue adhesive and the animals were observed during recovery.

2.3. Measurements of breathing patterns

Respiratory waveforms were recorded from spontaneously breathing rats in a temperature-equilibrated whole-body plethysmograph following an acclimatization period. Pressure changes in the chamber were passed through a pre-amplifier (Max II, Buxco Electronics), acquired (Sampling rate = 200 Hz, Power1401, CED, Cambridge, UK) and stored with respiratory acquisition software (Spike 2, CED) for off-line analysis of breathing-pattern dynamics.

2.4. Assessment of lung injury

At the 48 h time point, the rats were sacrificed by an over dose of anesthetic and their chest cavity opened (Jacono et al., 2006). A tracheal cannula was secured and the lungs lavaged with saline (2× with 2.5 ml each time). Protein concentration of BALF supernatant was measured using a conventional dye-binding assay (Bio-Rad Laboratories, Hercules, CA) and analyzed spectrophotometrically. The BALF cell pellet was resuspended in PBS and viable cells were identified by Trypan blue exclusion and counted by a reader blinded to the injury status of the animal. In other animals, lungs were inflated and fixed with 10% formalin at 25 cm H_2O for 30 min. The lungs were then removed *en bloc*, transferred to a cassette, and embedded in paraffin. Subsequently, 5-μm sections were cut and stained with hematoxylin and eosin for histological examination.

2.5. Lung tissue and serum cytokine levels

Unfixed lung tissue was homogenized in complete lysis buffer and centrifuged. Cytokine concentrations (IL-1β, IL-6 and TNF-α) were measured in supernatants of homogenized lungs by quantitative sandwich enzyme immunoassay technique (ELISA) (R&D Systems). Standards and samples were pipetted into microplate wells pre-coated with a monoclonal antibody specific for the relevant cytokine. After washing, an enzyme-linked polyclonal antibody specific to relevant cytokine was added. After washing, substrate solution was added and the amount of bound cytokine quantified spectrophotometrically.

2.6. Brainstem cytokine assessment

2.6.1. Brainstem tissue removal

Animals were perfused transcardially with saline followed by 4% paraformaldehdye. The brainstems were removed, postfixed in 4% paraformaldehyde for 4 h at 4 °C, and cryoprotected in 15% sucrose overnight and then 30% sucrose until the tissue sank. Brainstems were then embedded in tissue freezing medium (Triangle Biomedical Sciences) and frozen sections (20 μm) were cut using a cryostat (Leica). All sections were collected on a series of 4 duplicate slides and stored at −20 °C until use.