Neuroscience Letters 671 (2018) 13–18

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Mechanical ventilation modulates pro-inflammatory cytokine expression in spinal cord tissue after injury in rats

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ARTICLE INFO

Keywords:
Spinal cord injury
Mechanical ventilation
Interaction
Inflammation
Healthy lung

ABSTRACT

Rationale: Spinal cord injury (SCI) may induce significant respiratory muscle weakness and paralysis, which in turn may cause a patient to require ventilator support. Central nervous system alterations can also exacerbate local inflammatory responses with immune cell infiltration leading to additional risk of inflammation at the injury site. Although mechanical ventilation is the traditional treatment for respiratory insufficiency, evidence has shown that it may directly affect distant organs through systemic inflammation.
Objectives: This study aimed to better understand the impact of invasive mechanical ventilation on local spinal cord inflammatory responses following cervical or thoracic SCI.
Methods: Five groups of female Sprague-Dawley rats were anesthetised for 24 h. Three groups received mechanical ventilation: seven rats without SCI, seven rats with cervical injury (C4-C5), and seven rats with thoracic injury (T10); whereas, two groups were non-ventilated: six rats without SCI; and six rats with thoracic injury (T10). Changes in inflammatory responses were determined in the spinal cord tissues collected at the local site of injury. Cytokines were measured using ELISA.
Main results: SCI induced local pro-inflammatory cytokine IL-6 expression for all groups. Mechanical ventilation also had effects on pro-inflammatory cytokines and independently increased TNF-α and decreased IL-1β levels in the spinal cords of anesthetized rats.
Conclusion: These data provide the first evidence that mechanical ventilation contributes to local inflammation after SCI and in the absence of direct tissue injury.

1. Introduction

A spinal cord injury (SCI) is a devastating neurological insult that can result in lifelong disability, accompanied by increased morbidity and mortality [25]. Patients with higher cervical injuries often develop respiratory insufficiency and require mechanical ventilation (MV) to compensate for respiratory muscle weakness or paralysis [37]; patients with lower thoracic lesions may also temporarily require MV. There is evidence that MV can contribute to the development of ventilator induced lung damage not only in previously injured lungs but also in healthy lungs in both animals and humans [36]. Aside from directly affecting the lungs, studies have also demonstrated that MV can trigger the local release of such inflammatory cytokines as tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and -1β [4]. Additional evidence suggests that MV applied to healthy lungs may induce a systemic inflammatory response [26].

Acute SCI provokes massive immune cell recruitment locally, associated with an inflammatory cellular response that results in tissue damage at the injury site [8,10]. When the spinal cord is injured, neutrophils and macrophages (also known as microglia), components of the first line of immune defense, are mobilized and activated in large numbers early post-trauma in order to clean up the injured tissue zone of cell debris [2]. These immune cells release pro-inflammatory mediators such as reactive oxygen species and oxidative enzymes [19].

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https://doi.org/10.1016/j.neulet.2018.01.028
Received 18 November 2017; Received in revised form 15 January 2018; Accepted 15 January 2018
Available online 31 January 2018
0304-3940/

Macrophages are a source of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) as well as oxidative stress [16]; they may intensify early local inflammatory responses leading to secondary spinal tissue damage [15] but can also have anti-inflammatory properties which can promote tissue repair and axonal regeneration [1]. The increased inflammatory responses contribute to the proliferation of resident cells (astrocytes and microgliocytes) at the local junction between healthy and damaged tissues, thus contributing to astroglial scarring [27]. However, these activated microglia cells are believed to play detrimental roles during the inflammatory response following a SCI, with the inhibition of tissue repair and axonal neuroregeneration [12]. In addition, more recent studies have shown that the anti-inflammatory environment could promote functional recovery after SCI [18,29]. The exacerbation of initial tissue damage or promotion of repair in central nervous system (CNS) will depend preferentially on a pro-inflammatory *vs* anti-inflammatory environment, respectively, and the type of cytokine induction that occurs [17,33].

A systemically induced pro-inflammatory phenomenon could potentially participate in a distant unbalanced repair of the spinal cord tissue. However, the understanding of the inflammatory responses induced by the application of MV in the context of an acute spinal cord injury and how these affect the local spinal cord inflammation is currently unknown. We hypothesized that MV would contribute to a pro-inflammatory environment and modify inflammatory patterns at the level of the injured spinal cord tissue. The purpose of the present study was to determine the impact of 24 h of MV on local spinal cord inflammatory responses after injury.

2. Material and methods

2.1. Study approval

All procedures were conducted according to the recommendations of the Canadian Council for Animal Care and were approved by the Animals Committee of the Research Center of Sacré-Coeur Hospital of Montreal.

2.2. Study design

The present study was a comparative study with and without exposure to mechanical ventilation, following cervical and thoracic SCI in rats. The primary outcome was the pattern of the local inflammatory responses determined by measuring cytokine levels.

2.3. Animal preparation

Forty-six adult female Sprague-Dawley rats (225–250 g; Charles River, St Constant, Quebec, Canada) were used for the study. Prior to experiments, the animals were housed pairwise per cage, with free access to food and water. Thirteen died within 24 h of the study and thirty-three were included in the analysis after experiments. Animals were randomized into five separate groups by drawing lots. Two groups received no MV whereas three others did. For the rats without MV support, one group had no SCI (N = 6) and the other had a thoracic lesion (TL) (N = 6, T10). For the ventilated rats, one group had a cervical lesion (CL) (N = 7, C4-C5), the second group had a TL (N = 7, T10) and the third group had no SCI (sham, N = 7). The experiments were carried out in the surgical animal facility and initiated in the morning. The rats were first anesthetized with ketamine/xylazine (90/10 mg/kg, i.m) after which each animal received an adjusted dosage of the same two agents administered continually over 24 h and delivered *via* catheter placed in the jugular vein. All animals were placed on a heating blanket for 24 h to prevent a decrease in body temperature. Body temperature was continuously monitored and maintained between 37 °C and 39 °C. Heart rate was monitored using subcutaneous electrodes (Nihon Kohden, Tokyo, Japan) in the paw of the animals. All animals received the following procedures every 4 h: 1) mobilization of the extremities to compensate for the loss of venous return, and 2) manual massaging of the bladder to prevent renal stasis.

2.4. Mechanical ventilation

In ventilated animals, ventilator support was provided before each laminectomy (spinal cord injury or sham) *via* tracheal intubation with an endotracheal tube secured in place using surgical thread. The ventilator settings (Kent Scientific, Topo, Torrington, CT) were maintained for 24 h to provide an appropriate tidal volume (Vt = 6 mL/kg) based on the animal's body weight according to the nomogram of Kleiman and Radford [14] with a constant: 1) peak inspiratory airway pressure (PIP) of 10 cm H_20, 2) respiratory rate (RR) 60 breaths per minute, 3) inspiration-to-expiration ratio of 1:3 achieved by setting the inspiration % at 35%, and 4) expired end-tidal $C0_2$ between 2.5 and 3.5% (Capstar-100 CO_2 analyzer). For non-ventilated animals, supplemental oxygen was given (0.1 L/min) through an adjusted facial mask.

2.5. Spinal cord injury

The SCI procedure was performed as described Dery et al. [7]. A laminectomy was performed at C4 or T9 vertebrae, exposing the C5 or T10 spinal cord segment. The stereotactic clamps were installed on both sides of the laminectomy to stabilize the spine of the injured animals. To produce a moderate SCI, a weight of 5 g from a height of 6 cm was dropped directly onto the spinal cord (30 g cm impact). For animals with no SCI, the same surgical procedures were performed except for the laminectomy and impact to the spinal cord. The muscles and skin were then sutured. Finally, an eye gel containing white Vaseline and mineral oil respectively (80% and 30%, Duo Lube, Bausch & Lomb, Markham, Canada) was used to prevent xerophtalmia due to anesthesia. Anesthetized rats were euthanized by exsanguination 24 h post-injury.

2.6. Bronchoalveolar lavage and collection of tissues samples

A bronchoalveolar lavage procedure was performed as described previously by Truflandier et al. [34]. A tracheotomy was performed to insert an endotracheal tube, secured in place using surgical thread, into the trachea of rats which did not previously receive MV. In intubated rats, the tube already in place in the trachea was used. Then, in order to proceed to a bronchoalveolar lavage (BAL), the thorax was opened, the right lung clamped at the bronchus level, and the left lung was subjected to five consecutive lavages through the tracheal tube. Each BAL was performed by introducing 2.5 mL of filtered sterile phosphate-buffered saline (PBS) containing 0.5% of bovine serum albumin (2.5 mL PBS/BSA) into the tube extremity and drawn up by a 5 mL syringe placed at the other extremity. The fluid recovered from the first wash was injected into a 15 mL falcon tube, while the remaining four subsequent washes where transferred to a second separate falcon tube. Both tubes were placed on ice in order to keep cells alive and to be processed for measurement of total cell counts, inflammatory mediators, and oxidative stress status. The material obtained from the first wash was centrifuged at 350*g* for 10 min at 4 °C and the supernatant was harvested and aliquoted in Eppendorf tubes kept at 80 °C until assessment of pulmonary analysis. Finally, spinal cord injured tissues, diaphragm and right lung were removed and kept intact, immersed in liquid nitrogen before being frozen at −80 °C for future analyses.

2.7. Protein assay and determination of inflammatory cytokine concentrations

Spinal cord tissues, which were collected at the injury site, were thawed before measuring inflammatory cytokines. First, a protein assay was done by standard Bradford Assay Method [7]. To obtain the mass of spinal cord samples, spinal tissues (30–40 mg) were transferred into