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Basic Science

Pulmonary edema and hemorrhage after acute spinal cord injury in rats

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Abstract

BACKGROUND CONTEXT: Respiratory complications are a major cause of morbidity and mortality during the first days after acute spinal cord injury (ASCI). However, the pathophysiology of respiratory insufficiency resulting from spinal cord injury that involves lower levels is less well understood.

PURPOSE: The aim of the present study was to investigate pulmonary pathophysiology after ASCI.

STUDY DESIGN: This is an experimental animal study of ASCI investigating pulmonary pathophysiology after ASCI.

METHODS: Eighty-four (N=84) rats were divided into two groups: a sham surgery (n=42) and an injury group (n=42). In the injury group, ASCI was induced at the level of the tenth thoracic vertebra by a modified Allen method. Rats were sacrificed 6 hours, 12 hours, 24 hours, 3 days, 1 week, 2 weeks, and 4 weeks after surgery. Pulmonary edema was assessed by calculating the ratio of the wet-to-dry lung weight (W:D). Pulmonary edema and hemorrhage were evaluated by observing gross and microscopic morphology. The study was funded by Natural Science Foundation of China (NSFC, 81272172). The funder of the present study had no capacity to influence the scholarly conduct of the research, interpretation of results, or dissemination of study outcomes.

RESULTS: In the injury group, W:D was significantly increased 12 hours after surgery compared with the sham surgery group; W:D peaked 3 days after ASCI ($p<.05$). Gross morphologic observations showed hemorrhagic lesions on the lung tissue 12 hours after ASCI and pulmonary edema 24 hours after ASCI. Pulmonary edema peaked 3 days after ASCI and was obviously decreased 1 week after ASCI. Hemorrhage was apparent until 2 weeks after ASCI. Light microscopy showed congestion of pulmonary capillaries 6 hours after ASCI. The pulmonary alveoli were filled with erythrocytes and serous extravasate 12 hours after ASCI. Hemorrhage and edema were observed in the interstitium and lung alveoli 24 hours after ASCI.

CONCLUSIONS: Early pathologic changes such as pulmonary congestion, hemorrhage, and edema after injury may be the basis for early respiratory dysfunction following ASCI.

Keywords: Edema; Hemorrhage; Pulmonary; Rat; Spinal cord injury; neurogenic pulmonary edema

Introduction

Respiratory complications are a major cause of morbidity and mortality during the first days after acute spinal cord injury (ASCI) [1–3]. The incidence of respiratory complications in ASCI varies from 36% to 83%, and respiratory complications during the acute hospital stay contribute significantly to health-care costs [4].

Pulmonary edema, alveolar hypoventilation, pneumonia, alterations in respiratory mechanics, pulmonary embolism, and aspiration of gastric contents are common respiratory complications, especially in high-level ASCI [1]. In the clinic, generally speaking, the main causes of respiratory complications are pulmonary embolism, respiratory muscle dysfunction, and reduced ability to cough [5].

It is well known that cervical spine lesions above the C3 level lead to complete paralysis of all muscles involved

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with respiration [1]. However, the pathophysiology of respiratory insufficiency resulting from spinal cord injury that involves lower levels is less well understood. Recent studies suggest a role for neurogenic pulmonary edema (NPE). Neurogenic pulmonary edema is an acute life-threatening complication of severe central nervous system (CNS) injury with unknown etiopathogenesis [6,7]. Previous reports suggest that NPE is characterized by marked pulmonary vascular congestion, extravasation of protein rich fluid, edema, and intra-alveolar hemorrhage [2]. Studies in a rat model from our laboratory indicate that NPE and severe pulmonary hemorrhage after ASCI are associated with significant mortality [8].

Reports on the pathogenesis of NPE following ASCI are limited [9]. Therefore, the aim of the present study was to evaluate NPE, hemorrhage, and pulmonary pathology following ASCI induced by a modified Allen method in the rat.

Materials and methods

Animals

Eighty-four (N=84) adult Wistar rats (male and female; [1:1]; weight: 240–250 g; age: 6–8 weeks) were provided by the Animal Center of Research Institute of Surgery of the Third Military Medical University of Chinese PLA. Rats were randomly divided into either a sham surgery (n=42) or injury (n=42) group. We used coin flipping to decide which rat belongs to which group. This ensures that each rat has an equal chance of being placed in any group. Random assignment of participants helps ensure that any differences between and within the groups are not systematic at the outset of the experiment.

The present study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, China, and the Experimental Animal Committee of the Chongqing Medical University (Permit numbers: SCXK [Yu] 2012 – 0001 and SYXK [Yu] 2012 – 0001). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were done to minimize suffering.

Based on our statistical estimation and previously published papers, six rats were used per group per time point. In the injury group, ASCI was induced at the level of the tenth thoracic vertebra by a modified Allen method. Six rats from the injury and sham groups were sacrificed 6 hours, 12 hours, 24 hours, 3 days, 1 week, 2 weeks, and 4 weeks after surgery.

On the first postoperative day, the behavior of the rats was assessed and respiration was monitored once every 4 hours. Thereafter, the behavior of the rats was assessed and respiration was monitored once every 12 hours. During the acute phase post injury, rats were evaluated by checking bladder size, sutures, and body weight twice a day. The bladder was gently squeezed (twice a day for 7 days) to avoid urinary tract infections (defined as urine that was cloudy, bloody, or containing any precipitates).

After spinal cord injury, some rats showed self-destructive behavior and attacked each other. We isolated the rats that were kept separately. If the rats continued to show self-destructive behavior, they were euthanized using CO_2. If the rats were sacrificed, we added the same condition rats model in the same group to continue the study. We ensured each group has 42 rats at the end of the study. Throughout the study period, the rats were checked for injuries and their ability to feed. Artificial feeding was used as required.

Allen method for spinal cord lesions

Before skin preparation and routine sterilization, rats were intraperitoneally anesthetized using 10 g/L sodium pentobarbital (40 mg/kg) and were fixed on an operating table. A 2.0-cm incision around the T_{10} lamina exposed and separated the spinal process. The erector spinae was exposed and the T_{10} lamina was removed, with the exception of the dura mater. In the sham surgery group, the spinal cord was exposed without contusion. In the injury group, a modified Allen method was used to induce contusion injury [10]. Briefly, a thin copper slice was used to cover the exposed spinal cord, which was vertical to a graduated glass tube. A 10-g metal block was dropped onto the copper slice from a height of 2.5 cm. The copper slice was immediately removed. If a rat's tail curved or swung, injury was considered successful.

Following surgery, all rats were injected intraperitoneally with penicillin (20,000 U/kg) twice per day for three consecutive days. Subsequently, the rats were housed in an air-conditioned room and were allowed to freely feed. Forced urination was performed three times a day.

Evaluation of pulmonary edema and hemorrhage

The rats (n=6) from each group were sacrificed at various time points, ranging from 6 hours to 28 days (6 h, 12 h, 24 h, 3 d, 1 wk, 2 wk, and 4 wk) after surgery. The rats were perfused with 4% paraformaldehyde. At each time point, the lungs of six rats were immediately removed and weighed to obtain the wet weight (W). After drying in the oven, the lungs were weighed again to obtain the dry weight (D). Edema was assessed by calculating the ratio of the wet-to-dry lung weight (W:D).

In addition, the lungs of the rats were removed, fixed immediately in 4% paraformaldehyde in phosphate buffer (pH 7.4) for 2 days, and embedded in paraffin. Tissue was cut into 5-μm thick sections and stained with hematoxylin and eosin to observe changes in pulmonary pathophysiology. At each time point, the lungs of six rats from each group were removed and two pieces of paraffin sections were made of each rat. So every group has 12 pieces of paraffin sections at each time point. Histologic scoring was calculated under high-power (400×) magnification at three randomly selected sites. The pathologist was blinded to the injury and sham groups. Histologic scoring based on categories of infiltration of inflammatory cells, edema, congestion, and intra-alveolar