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Behavioral and anatomical consequences of repetitive mild thoracic spinal cord contusion injury in the rat

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ABSTRACT

Moderate and severe spinal cord contusion injuries have been extensively studied, yet much less is known about mild injuries. Mild contusions result in transient functional deficits, proceeding to near-complete recovery, but they may render the spinal cord vulnerable to future injuries. However, to date there have been no appropriate models to study the behavioral consequences, anatomical changes, and susceptibility of a mild contusion to repeated injuries, which may occur in children as well as adults during competitive sport activities. We have developed a novel mild spinal cord contusion injury model characterized by a sequence of transient functional deficits after the first injury and restoration to near-complete motor and sensory function, which is then followed up by a second injury. This model can serve not only to study the effects of repeated injuries on behavioral and anatomical changes, but also to examine the relationship between successive tissue damage and recovery of function. In the present study, we confirmed that mild thoracic spinal cord contusion, utilizing the NYU impactor device, resulted in localized tissue damage, characterized by a cystic cavity and peripheral rim of spared white matter at the injury epicenter, and rapid functional recovery to near-normal levels utilizing several behavioral tests. Repeated injury after 3 weeks, when functional recovery has been completed, resulted in worsening of both motor and sensory function, which did not recover to prior levels. Anatomical analyses showed no differences in the volumes of spared white matter, lesion, or cyst, but revealed modest extension of lesion area rostral to the injury epicenter as well as an increase in inflammation and apoptosis. These studies demonstrate that a mild injury model can be used to test efficacy of treatments for repeated injuries and may serve to assist in the formulation of policies and clinical practice regarding mild SCI injury and spinal concussion.

Introduction

Injuries to the spinal cord result in functional deficits, which depend on the severity and level of injury. Various animal models for spinal cord injury (SCI) including contusion, compression, transection, and hemisection have been developed and used depending upon the experimental goals of the study. Among these injuries, contusion injuries are the most common in human SCI. Contusion lesions created by a computer controlled impactor can be designed to be mild, moderate, or severe (Basso et al., 1996). While moderate and severe contusion injuries have been extensively studied, much less is known about the consequences of mild injuries. A mild injury, clinically defined as a spinal cord concussion, results in transient neurologic disturbances with deficits in sensory and motor function. A complete neurological recovery usually occurs within two to three weeks in animal mild contusion models (Basso et al., 1995, 1996; Scheff et al., 2003; Zhang et al., 2008), but the long term consequences of the injury are not known. Importantly, there is a long clinical history of spinal cord concussion, though it has assumed a variety of clinical terms, including transient paraplegia/quadriplegia/paresis, transient traumatic paraplegia/quadriplegia, neurapraxia, and spinal cord concussion (Brigham and Capo, 2013; Cantu and Cantu, 2005; Maroon et al., 2007; Torg et al., 1997; Torreman et al., 1996; Winder et al., 2011; Zwimpfer and Bernstein, 1990). Both pediatric and adult patients experience transient motor and sensory dysfunction following an acute blow to the cervical or thoracic spinal cord that gradually resolves over a period of 10 min to 48 h, without any radiological abnormalities observed within the spinal cord itself. Most patients sustain injuries following participation in a diverse array of contact sport activity, including American football, rugby, hockey, and wrestling, with the highest incidence rate occurring in American football — approximately 1.3 cases per 10,000, though this likely represents a significant underrepresentation due to failed reporting (Torg et al., 1986). Repeated spinal cord concussions are well known to occur, and numerous reports have documented increased recovery times, progressive neurological damage, and complete paralysis upon secondary concussion.

Unlike spinal cord concussion, brain concussions (mild traumatic brain injuries, mTBIs) have been studied extensively, as hundreds of thousands of sport- and combat-related injuries occur each year

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(Dietrich et al., 1994; Johnson et al., 2010; Povlishock et al., 1983; Prins et al., 2010). Although mTBI is usually not life threatening, their effects can have serious consequences. People who have had one concussion are more susceptible to another, especially if the new injury occurs before symptoms from the previous concussion have been completely resolved (Gronwall and Wrightson, 1974). In addition, there is also a negative progressive process in which smaller impacts cause the same symptom severity. Repeated concussions may also increase the risk in later life for dementia, Parkinson's disease, and/or depression (Mannix et al., 2013; Plassman et al., 2000). Animal studies indicate a complex pathology that includes the disruption of neuronal cell membrane accompanied by release of glutamate and a lower metabolic state which may persist for weeks after injury (Giza and Hovda, 2001). The efforts to increase awareness about symptoms of mTBI and how to manage them have culminated in the Zurich Consensus Statement on Concussion in Sport, which recommends persons to be symptom free before restarting activity, and then not all at once, but rather through a series of graded steps (McCrory et al., 2009).

Thus far, there have been no comparable studies on the effects and risks of repeated mild SCI in animal models. For example, it is not clear whether repeated mild contusion SCI results in cumulative or synergistic effects, with detrimental consequences on the potential for functional recovery, and whether the increased vulnerability is associated with specific biochemical, immunological, or histological changes. In this study, we addressed these issues in an established animal model, mild spinal cord contusion using the NYU-MASCIS (New York University — Multicenter Animal Spinal Cord Injury Study) impactor. Our findings indicate that even when the second injury occurs following the completion of recovery, the resulting deficits in both motor and sensory functions worsen, and there is no longer recovery to the same levels as the first injury.

Materials and methods

Animals

Female Sprague–Dawley rats (225–250 g) were obtained from Taconic Farms (Germantown, NJ). They were housed 3 per cage with a 12 h light/dark cycle. Food and water were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Drexel University College of Medicine and were carried out according to the *NIH Guide for the Care and Use of Laboratory Animals*.

Surgical procedure

Twenty five rats received a mild contusion and were divided into two groups three weeks post-injury, after complete recovery (defined as an average BBB score of 19): animals that received one contusion (C1, n = 12), and animals that received a second contusion (C2, n = 13).

After intraperitoneal (i.p.) administration of anesthesia – XAK, a mixture containing xylazine (10 mg/ml), acepromazine maleate (0.7 mg/kg), and ketamine (95 mg/kg) – a laminectomy was performed at T10, and a contusion lesion was made with the NYU-MASCIS impactor (10 g weight dropped from a height of 6.25 mm). Following injury, the muscle and skin were closed in layers. Three weeks after contusion, animals were re-anesthetized with XAK and divided into two groups: C1: control animals where the lesion site was reopened, and C2: experimental animals where the lesion was re-opened and followed by the same mild contusion as in the first injury. Rats were placed back in their cages with heating pads, and closely observed until they awoke. Saline was injected subcutaneously immediately after lesion and then daily for 7 days. Buprenex (0.015–0.02 mg/kg, 0.3 mg/ml, Reckitt Benckiser, Richmond, VA) was administered subcutaneously post-surgery and twice a day for two days. Ampicillin (Bristol-Myers Squibb, Princeton, NJ) was injected daily for 7 days postoperatively. Bladders were manually expressed twice a day for 1–2 weeks and then once a day until full recovery.

Evaluation of motor and sensory function

Three behavioral tests were performed to evaluate motor and sensory changes, including open-field locomotion (Basso Beattie Bresnahan scale, BBB), grid, and Catwalk. A total 25 rats were examined utilizing the BBB scale. The grid test was added to the functional analyses after the first 5 rats received their second set of surgeries, therefore these rats were excluded from grid test analysis due to the lack of baseline data. A total of 20 rats (a subset of the 25 receiving BBB) were analyzed using the grid test. Due to the availability of Catwalk instrument, only the last 14 rats (a subset of the 25 receiving BBB) received Catwalk analysis. One rat was removed from Catwalk analysis due to abnormal baseline step pattern. Therefore, 13 rats were included in the Catwalk analyses. All behavioral analyses were made by observers blinded to the experimental groups.

Open-field locomotion (BBB)

Rats were acclimated to behavioral apparatus for one week prior to the first surgery. Rats were placed in an enclosure and scored by 2 blinded observers according to the BBB scale (Basso et al., 1995). Rats were scored prior to contusion injury to establish a baseline, 2–3 days after 1st contusion, 2nd contusion, and control animals that underwent sham contusion. The analysis continued once a week until the end of the experiments. A total of 25 rats (C1, n = 12; C2 = 13) were evaluated by the BBB.

Grid test

Rats walked on an elevated grid (36L × 38W × 30H cm) with 1.2 × 1.2 cm openings for 2 min. The total number of correct steps over the total number of steps was calculated for each hindpaw. Grid tests were performed weekly on animals with a BBB score above 10. A total of 20 rats (C1, n = 10; C2, n = 10) were evaluated by the grid test.

Catwalk gait analysis

Animals crossed a Plexiglas floor walkway, which allowed for the visualization of foot contacts, and a high speed camera recorded the animal runs in a dark room (Hamers et al., 2001; Hamers et al., 2006). Paw placement during locomotion was analyzed using Catwalk software NXT 10 (Noldus Information Technologies). Animals were pre-trained for 5 days prior to the first surgery and tested every week. Base of support, paw angles, step sequence, and phase dispersion were analyzed. A total of 13 rats (C1, n = 7; C2, n = 6) were evaluated by Catwalk analysis.

Tissue preparation

Animals were anesthetized with i.p. injections of sodium pentobarbital (100 mg/kg, Abbot Laboratories, North Chicago, IL) and transcardially perfused with 100 ml saline followed by 500 ml of ice-cold 4% paraformaldehyde fixative in phosphate buffer (PB, pH 7.4). A 30 mm long segment including the lesion area was dissected. Tissues were post-fixed in the same fixative for 2–3 days and then transferred into a solution of 30% sucrose in 0.1 M PB for 3–5 days. Spinal cord segments were embedded in M1 mounting medium (Fisher Scientific, Pittsburgh, PA). The 30 mm long lesion-containing segments were cut into 20 μm coronal sections on a cryostat, and mounted onto gelatin-coated slides in 10 serial sets. After harvesting the first 20 μm section, the subsequent four sections were discarded, prior to harvesting another section, making the interval between sections on the same slide 1 mm.