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Regular Article

Evaluation of the anatomical and functional consequences of repetitive mild cervical contusion using a model of spinal concussion

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ABSTRACT

Spinal cord concussion is characterized by a transient loss of motor and sensory function that generally resolves without permanent deficits. Spinal cord concussions usually occur during vehicular accidents, falls, and sport activity, but unlike brain concussions, have received much less attention despite the potential for repeated injury leading to permanent neurological sequelae. Consequently, there is no consensus regarding decisions related to return to play following an episode of spinal concussion, nor an understanding of the short- and long-term consequences of repeated injury. Importantly, there are no models of spinal concussion to study the anatomical and functional sequelae of single or repeated injury. We have developed a new model of spinal cord concussion focusing on the anatomical and behavioral outcomes of single and repeated injury. Rats received a very mild (50 kdyn, IH impactor) spinal contusion at C5 and were separated into two groups three weeks after the initial injury — C1, which received a second, sham surgery, and C2, which received a second contusion at the same site. To track motor function and recovery, animals received weekly behavioral tests — BBB, CatWalk™, cylinder, and Von Frey. Analysis of locomotor activity by BBB demonstrated that rats rapidly recovered, regaining near-normal function by one week after the first and second injury, which was confirmed using the more detailed CatWalk™ analysis. The cylinder test showed that a single contusion did not induce significant deficits of the affected limb, but that repeated injury resulted in significant alteration in paw preference, with animals favoring the unaffected limb. Intriguingly, Von Frey analysis demonstrated an increased sensitivity in the contralateral hindlimb in the C2 group vs. the C1 group. Anatomical analyses revealed that while the lesion volume of both groups was minimal, the area of spared white matter in the C2 group was significantly reduced 1 and 2 mm rostral to the lesion epicenter. Reactive astrocytes were present in both groups, with the majority found at the lesion epicenter in the C1 group, whereas the C2 group demonstrated increased reactive astrocytes extending 1 mm caudal to the lesion epicenter. Macrophages accumulated within the injured, dorsal and ipsilateral spinal cord, with significant increases at 2 and 3 mm rostral to the epicenter in the C2 group. Our model is designed to represent the clinical presentation of spinal cord concussion, and highlight the susceptibility and functional sequelae of repeated injury. Future experiments will examine the temporal and spatial windows of vulnerability for repeated injuries.

1. Introduction

Concussion is a temporary disruption of neuronal function caused by traumatic biomechanical forces, which initially was thought to result in neuronal, chemical, or neuroelectrical changes without significant structural damage (Cantu, 1996). Spinal cord concussion is a variant of mild cord injuries that results in transient neurologic disturbances, associated motor and sensory loss, and complete recovery within 48–72 h after injury (Del Bigio and Johnson, 1989; Zwimpfer and Bernstein, 1990). Spinal cord concussion has assumed a variety of clinical designations, including transient paraplegia, transient traumatic paraplegia/quadriplegia, transient paraplegia/quadriplegia/paresis, and neurapraxia (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). Patients who suffer spinal cord concussion exhibit a stereotypic clinical syndrome: transient motor and sensory loss immediately preceded by an acute blow to the spinal cord that gradually resolves without additional intervention. Spinal cord concussion occurs across a wide variety of sports in both professional and amateur adult and pediatric athletes (Rathbone et al., 1992) — wrestling (Bailes, 2005; Rathbone et al., 1992), rugby (Scher, 1991), American football (Cantu and Cantu, 2005; Torg et al., 1986; Winder et al., 2011), hockey (Winder et al., 2011), gymnastics (Rathbone et al., 1992), and diving (Rathbone et al., 1992). Importantly, many individuals have experienced multiple episodes of spinal cord concussion (Brigham and Capo, 2013; Cantu and Cantu, 2005;

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Gronwall and Wrightson, 1974; Maroon et al., 2007; Torg et al., 1997; Torreman et al., 1996; Winder et al., 2011; Zwimpfer and Bernstein, 1990). In addition, spinal cord concussions have also been reported to occur after a variety of non-sporting activities, such as car accidents and falls (Zwimpfer and Bernstein, 1990).

Unlike brain concussion, or mild traumatic brain injury (mTBI), there have been no appropriate models to study the effects and risks of spinal cord concussion. Studies on brain concussion have revealed that individuals who suffered a single concussion are more susceptible to another, especially if the new injury occurs before symptoms from the previous concussion have completely resolved (Gronwall and Wrightson, 1974). In addition, there is also a negative progressive process in which smaller, subsequent impacts cause the same symptom severity as the initial injury. Moreover, repeated concussions may increase the risk in later life for dementia, Parkinson's disease, and/or depression (Mannix et al., 2013; Plassman et al., 2000). Animal studies have indicated that concussion effects a complex pathology that includes the disruption of neuronal cell membranes 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 education about symptoms of mTBI and how to manage them are exemplified by the 2008 Zurich Consensus Statement on Concussion in Sport, which recommends persons to be symptom free before restarting and then, not all at once, but rather through a series of graded steps (McCrory et al., 2009).

In contrast, relatively little was known about the consequences and long-term sequelae of spinal cord concussion at its outset. There are no answers to the questions related to 1) when can athletes return to play after spinal cord concussion and 2) what are the consequences of the repeated spinal cord concussions that athletes suffer. So far, no experimental models have been developed or data on repeated spinal cord concussion reported. Our previous study (Jin et al., 2014) on repetitive mild SCI at the thoracic level indicated that functional deficits worsened with a second injury and resulted in increased tissue damage, inflammation, and cell death. However, the established "mild SCI" produced by the NYU impactor in that study resulted in massive tissue damage at the lesion epicenter and a slow functional recovery that returned to near-normal levels only by three weeks post-injury. This mild SCI model (Jin et al., 2014) therefore does not faithfully reproduce the clinical presentation of spinal cord concussion, which is characterized by minimal tissue damage and rapid functional recovery. In the present study, we developed a new spinal cord concussion model using the Infinite Horizon (IH) impactor to examine the behavioral and anatomical consequences of single and repeated injury. We also compared this new, very mild spinal cord concussion model (referred to as minimal contusion) with the standard mild thoracic contusion we have previously studied (Jin et al., 2014).

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats (225–250 g) were obtained from Taconic Farms (Germantown, NJ) and 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*.

2.2. Surgical procedure

Nineteen rats received a minimal contusion using a 50 kilodyne (kdyn) force by the IH impactor and were divided into two groups three weeks post-injury, after complete recovery (defined as an average BBB score around 21): animals that received one contusion (C1; n = 9), and animals that received a second contusion (C2; n = 10).

Rats received a mixture of XAK containing Xylazine (10 mg/ml), Acepromazine maleate (0.7 mg/kg), and Ketamine (95 mg/kg) injected intraperitoneally (i.p., volume/rat = body weight/rat × 0.00108 ml). The skin over the upper cervical area was shaved and cleaned with betadine solution and 70% ethanol. The skin and muscles were incised to expose the fifth cervical vertebral body (C5). A laminectomy was performed at C5, and a contusion was made on the right side of the cord with the IH impactor (Precision Systems and Instrumentation, LLC, Lexington, KY). Dwell time was set to 0 s. The IH device was set to deliver a force of 50 kydn. 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 delivered the same minimal contusion (50 kdyn) as in the first injury at the same location (C5). Rats were placed back in their cages with heating pads and closely observed until they awoke. Buprenex (0.015–0.02 mg/kg, 0.3 mg/ml, Reckitt Benckiser, Richmond, VA) was administered subcutaneously post-surgery and once a day after surgery for one day.

2.3. Evaluation of motor and sensory function

Baseline values for all behavior tests were acquired prior to the first surgery. All animals were then tested weekly.

2.3.1. Open-field locomotion (BBB)

The rats were placed in an enclosure and scored by two blinded observers according to the Basso, Beattie, and Bresnahan (BBB) rating scale (Basso et al., 1995). The test was performed prior to the initial contusion to establish a baseline, 2–3 days after the 1st and 2nd surgery, and once a week after surgery until the end of the experiment.

2.3.2. Paw Placement Test (cylinder test)

The rats were placed into a Plexiglas cylinder (17.8 cm by 35.5 cm). A mirror placed behind the cylinder allowed for observation of all movements. The test was video-recorded and the numbers of exploratory paw placements making contact with the cylinder were counted and categorized as left, right, or both paws. The score was expressed as a percentage of use of the affected limb (% of paw placements of the affected limb (right) + half of the % of both paws' placements divided by the total number of paw placements) (Liu et al., 1999; Shumsky et al., 2003).

2.3.3. CatWalk™ gait analysis

Animals crossed a Plexiglas floor walkway, which allowed for the visualization of foot contacts, and a high-speed camera recorded each of the animal runs in a dark room (Hamers et al., 2006; Hamers et al., 2001). Locomotion and gait was analyzed using CatWalk™ software NXT 10.5 (Noldus Information Technologies). Animals were pre-trained for 5 days prior to the first surgery and tested once a week until the end of the experiment. Base of support, step sequence, phase dispersion, swing, stance, and duty cycle were analyzed for locomotion changes. Mean intensity mean, maximum intensity mean, and maximum contact intensity were analyzed to describe sensory changes in all 4 limbs (Vrinten and Hamers, 2003).

2.3.4. Von Frey

The up–down method (Detloff et al., 2010, 2012) for Von Frey hair (VFH) monofilaments (VFH, Stoelting Co., Wood Dale, IL) was used to measure the degree of tactile sensory changes after minimal contusion. A total of 10 VFH stimulus applications were collected for each hind paw (Detloff et al., 2013). The response threshold was the lowest force (in grams) that produced a paw withdrawal in at least 50% of the applications. Paw testing order was determined randomly to minimize an order effect.