Neurobiology of Disease 105 (2017) 194–212

Contents lists available at ScienceDirect

Neurobiology of Disease

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

Generating level-dependent models of cervical and thoracic spinal cord injury: Exploring the interplay of neuroanatomy, physiology, and function

Jared T. Wilcox [a,b,1], Kajana Satkunendrarajah [a,1], Yasmin Nasirzadeh [a], Alex M. Laliberte [a], Alyssa Lip [a], David W. Cadotte [a,b], Warren D. Foltz [c], Michael G. Fehlings [a,b,*]

[a] Division of Genetics and Development, Toronto Western Research Institute, University Health Network, ON M5T 2S8, Canada
[b] Institute of Medical Science and Spinal Program, University of Toronto, ON M5S 1A8, Canada
[c] STTARR Innovation Centre, Department of Radiation Oncology, Princess Margaret Hospital, Toronto, ON M5G 1L7, Canada

ARTICLE INFO

Article history:
Received 9 March 2017
Revised 10 May 2017
Accepted 29 May 2017
Available online 31 May 2017

Keywords:
Spinal Cord Injury
Cervical
Thoracic
Clip compression
Magnetic resonance imaging
Forelimb
Gait analysis
Motor neuron survival
Principal component analysis
Discriminant function analysis

ABSTRACT

The majority of spinal cord injuries (SCI) occur at the cervical level, which results in significant impairment. Neurologic level and severity of injury are primary endpoints in clinical trials; however, how level-specific damages relate to behavioural performance in cervical injury is incompletely understood. We hypothesized that ascending level of injury leads to worsening forelimb performance, and correlates with loss of neural tissue and muscle-specific neuron pools. A direct comparison of multiple models was made with injury realized at the C5, C6, C7 and T7 vertebral levels using clip compression with sham-operated controls. Animals were assessed for 10 weeks post-injury with numerous (40) outcome measures, including: classic behavioural tests, CatWalk, non-invasive MRI, electrophysiology, histologic lesion morphometry, neuron counts, and motor compartment quantification, and multivariate statistics on the total dataset. Histologic staining and T1-weighted MR imaging revealed similar structural changes and distinct tissue loss with cystic cavitation across all injuries. Forelimb tests, including grip strength, F-WARP motor scale, Inclined Plane, and forelimb ladder walk, exhibited stratification between all groups and marked impairment with C5 and C6 injuries. Classic hindlimb tests including BBB, hindlimb ladder walk, bladder recovery, and mortality were not different between cervical and thoracic injuries. CatWalk multivariate gait analysis showed reciprocal and progressive changes forelimb and hindlimb function with ascending level of injury. Electrophysiology revealed poor forelimb axonal conduction in cervical C5 and C6 groups alone. The cervical enlargement (C5-T2) showed progressive ventral horn atrophy and loss of specific motor neuron populations with ascending injury. Multivariate statistics revealed a robust dataset, rank-order contribution of outcomes, and allowed prediction of injury level with single-level discrimination using forelimb performance and neuron counts. Level-dependent models were generated using clip-compression SCI, with marked and reliable differences in forelimb performance and specific neuron pool loss.

1. Introduction

Spinal cord injury (SCI) affects approximately 15,000 individuals each year in North America, leading to 1.2 million living with permanent deficits (Cripps et al., 2010; Sekhon and Fehlings, 2001; Singh et al., 2014; Wyndaele and Wyndaele, 2006). The degree of functional impairment depends on the level and severity of injury, and the resulting axonal tracts and regional circuitry affected. >60% of injuries to the spinal cord occur at the mid-cervical level, leading to loss of upper limb function (Farry and Baxter, 2010; Ghobrial et al., 2014; Sekhon and Fehlings, 2001).

By using preclinical studies that mimic the human condition and clinical needs, it may be possible to allow a more targeted approach to therapeutic studies. Return of hand function is the principal concern of persons with cervical spinal cord injuries, and represents the greatest impact on independence and quality of life (Anderson et al., 2005a; Lo et al., 2016; Tator, 1995). Recovery rates of arm and hand function is similar across different severities of cervical SCI, but is highly dependent on the level of injury (Steeves et al., 2007; van Hedel and Curt, 2006). Models of cervical injury currently represent a small proportion (2–12%) of all studies used to assess functional recovery

* Corresponding author at: Toronto Western Hospital, 399 Bathurst Street, ON M5T 2S8, Canada.
E-mail address: Michael.Fehlings@uhn.ca (M.G. Fehlings).
[1] Contributed equally to the work.
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2017.05.009
0969-9961/

(Antonic et al., 2013; Nielson et al., 2014), and clinical trials have largely been supported by rodent models of thoracic injury (Mack, 2011; Tetzlaff et al., 2011; Wilcox et al., 2012). Since the level of injury has a major impact on the extent of functional recovery (Kalsi-Ryan et al., 2013; Steeves et al., 2011; Wilson et al., 2012), and regaining neurologic/motor levels is a principal goal of therapeutics for SCI (Fawcett et al., 2007; Lammertse et al., 2012; Spiess et al., 2009; Steeves et al., 2011), studying how the level of injury relates to specific loss of function and neural components may provide valuable translational information.

Lesions to progressively rostral levels of the cervical spinal cord result in progressive and predictable motor deficits corresponding to the location of neurons that comprise motor pools that innervate specific muscles (Steeves et al., 2012). Cervical and thoracic spinal cord segments are neuroanatomically distinct, with appreciable differences in grey/white matter composition, the topography and organization of axonal pathways, and presence of limb-specific motor circuits. Cervical cord damage results in disruption to descending axonal pathways for below-level function, as well as loss of motor neurons supplying at-level function (Cote et al., 2012). The cervical enlargement, and specific neurologic levels that innervate specific muscles, is well preserved between humans and rats (McKenna et al., 2000; Tosolini and Morris, 2012). While ventral horn damage is known to determine forelimb deficit in the rat (Anderson et al., 2007; Pearse et al., 2005), few studies have determined the various neuronal populations at specific levels of the forelimb enlargement (Portiansky et al., 2004);(López-Dolado et al., 2012). Consequently, the loss of highly varied neuron populations and resulting functional deficits following injury has not been systematically characterized. However, being able to determine neurologic level of injury in preclinical models of SCI, and therefore if neurologic levels have been regained after therapeutic interventions, could significantly improve translation of preclinical studies.

To recapitulate human injury, forelimb function in preclinical models would exhibit progressive deficits with increased severity as well as higher neurologic level (Kalsi-Ryan et al., 2013; Takemi et al., 2014), which has proven difficult to date with rodent models (Anderson et al., 2004; Anderson et al., 2009a; Anderson et al., 2009b; Hilton et al., 2013; Takemi et al., 2014).

As more researchers are beginning to utilize cervical injury models (Anderson et al., 2009a; Anderson et al., 2009b; Dunham et al., 2010; Gensel et al., 2006; Iwasaki et al., 2014; Pearse et al., 2005; Soblosky et al., 2001; Wilcox et al., 2014), it may be useful to define the interplay of injury level and severity, and to determine which outcome measures identify variation between animals and groups in order to address the experimental objectives. Proper assessment of cervical injury may require different or novel outcome measures to appropriately determine recovery, preferably determined by unbiased analysis of large data sets (Ferguson et al., 2013).

To this effect, we sought to define the sensorimotor dysfunction, physiologic changes, and motor neuron loss in neurologically stable animals following severe clip compression injury to C5, C6, C7 and T7-injured animals. The study observes distinct and graded functional deficits between different levels of cervical and thoracic injuries, which have not been previously reported. Use of multivariate statistics identified the most relevant outcome measures for differentiating level of spinal cord injury. These outcomes include loss of total and cholinergic neuron populations, nerve conduction, detailed motor assessment using forelimb-specific tests, and gait analysis. Autonomic function, tissue volumes, and hindlimb-specific tests were not different between groups. Multivariate statistical analysis provided a unique ability to discriminate injury models to a single level using forelimb behaviour, gait performance, and neuron loss. From this study, we propose that the level of injury in severe bilateral cervical SCI can determine reliable behavioural and physiologic dysfunction due to level-specific neuroanatomical loss of distinct neuron populations.

2. Methods

2.1. Animals and surgery

All animal use, care, and experimental procedures were approved by the Animal Care Committee at the University Health Network in accordance with the policies of the Guide to the Care and Use of Experimental Animals as per the Canadian Council of Animal Care. Adult female Wistar rats ($n = 60$, 300–325 g, Charles River) were housed in pairs with free access to food and water, under 12 h light-dark cycle, at room temperature or 26 °C post-operative. The animals were divided into 5 groups, C5, C6, C7, T7 and sham ($n = 15, 15, 8, 8, 14$, respectively) (Table 1). A total of 46 rats underwent experimental spinal cord injury with a 28 g extra-dural clip compression of the spinal cord for 60 s at C6, C7, and T7, or 30 s for C5 as required by Animal Care Committee (Fig. 1). Humane euthanasia endpoints, exclusion criteria are described in detail in Supplemental Methods. Extensive and vigilant animal care by clinical veterinarians, care staff, and authors included manual bladder expression, hand-feeding, fluid adjuvant, and pain management, and contributed to overall low mortality rates in this severe high cervical injury model with mortality or exclusion not different between groups (Table 1).

2.2. Neurobehavioural analyses

Functional deficits caused by SCI at C5, C6, C7 and T7 vertebral levels of the spinal cord were determined by blinded examiners, with weekly neurobehavioural assessments and exercise to week 8, then CatWalk gait analysis and conduction physiology on week 10 prior to sacrifice (Supplemental methods).

2.2.1. Open field BBB

Basso, Beattie and Bresnahan (BBB) non-linear 22-pt rating scale was used for hindlimb locomotor function (Basso et al., 1995), with modified 14-pt BBB Subscore used to further assess limb movements during stepping (Ferguson et al., 2013).

2.2.2. Inclined plane

Global motor function was tested by animals' ability to sustain increasing degrees of incline with platforms ranging from 0° to 90°.

2.2.3. Grip strength

Forepaw and forelimb strength was tested with a grip strength meter with force transducer that measures the maximal weight generated when animals are pulled from a bar they have reflexively grasped.

2.2.4. Horizontal ladder walk

Paw placement and limb control was tested by counting the forelimb and hindlimb errors (slipping off or missing rungs) separately while animals crossed a narrow corridor with a floor comprised of unevenly placed rungs, maximal error or 12 per crossing.

2.2.5. F-WARP forelimb motor scale

A customized novel assessment scale (F-WARP: Fehlings and Wilcox Assessment of Rat Paw) graded forelimb muscle units on a 7-point scale irrespective of weight bearing, recording deficits in no muscle groups (6), digit abduction (5), distal digit flexion (4), proximal digital flexion and wrist flexion (3), wrist extension (2), pronation and internal rotation (1), shoulder abduction (0) (Suppl Table ST1).

2.2.6. CatWalk multivariate gait analysis

Paw and limb function was tested at endpoint using automated system (Noldus, Version 7.1) that measures static and dynamic parameters of locomotion while animals walk across a narrow corridor with glass floor. Video of each paw contact with the glass is recorded and quantified by software with low sensitivity threshold (25 au) for injured