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Tissue displacement and impact force are important contributors to outcome after spinal cord contusion injury

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Abstract

Spinal cord contusion injury in rodents is widely used as a model for spinal cord trauma in humans. Several biomechanical variables can influence injury outcome. In this work, we have assessed the influence of impact force and displacement of the spinal cord at the time of contusion injury on the severity of locomotor deficits and histopathological changes. Our work indicates that there is a linear relationship between force and tissue displacement, and that both these factors contribute to injury outcome. Furthermore, our work also suggests that setting narrow limits for the actual force applied (±5 kdyn) and tissue displacement (within a 200 μm range) will yield more consistent outcomes and provide greater sensitivity in detecting changes, regardless of the type of impactor device used.

Keywords: Spinal cord injury; Contusion; Macrophage; Myelin; Motoneuron; Locomotor function

Introduction

Spinal cord injury (SCI) in humans is often the result of a blunt trauma, followed by a period of tissue compression. This human pathology is best mirrored experimentally using animal models of contusion or compression injury (Gris et al., 2004; Jakeman et al., 2000; Joshi and Fehlings, 2002; Wrathall et al., 1985; Young, 2002). Contusion injury models are now commonly used to study SCI and its associated pathologies (Rosenzweig and McDonald, 2004; Scheff et al., 2003; Stokes and Jakeman, 2002; Young, 2002). This injury paradigm in the rodent results in the recruitment of immune cells, including neutrophils, macrophages, and T cells, at the site of injury. These inflammatory cells contribute to the reparative response (i.e., debris clearance and restoration of tissue integrity) but also become destructive, causing much of the secondary damage to axons and cells associated with SCI (Jakeman et al., 2000; Jones et al., 2002; Ma et al., 2002; Popovich et al., 1997; Sroga et al., 2003; Velardo et al., 2004). This pathophysiology results in motor and behavioral deficits. The similarities in the morphology and pathology of contused rodent spinal cords to that observed in the human (Bunge et al., 1993; Kakulas, 1999) suggest that this is a good model for the study of human SCI.

Three devices are currently widely used for the study of spinal cord contusion injuries in rodents. One of the earlier devices is the New York University impactor (Constantini and Young, 1994; Gruner, 1992), which is designed as a weight-drop model. In addition, other contusion devices also based on the weight-drop method are also used (Falconer et al., 1996; Farooque, 2000; Kuhn and Wrathall, 1998; Soblosky et al., 2001; Wrathall et al., 1985). The two newer contusion models, the Ohio State University Electromagnetic SCI Device [ESCID; (Jakeman et al., 2000; Stokes, 1992)] and the Precision Systems and Instrumentation Infinite Horizons [IH; (Scheff et al., 2003)] impactors, are displacement- and force-defined devices, respectively. All three devices can provide read-outs of tissue displacement, while the IH and ESCID devices can also measure the

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actual force delivered to the spinal cord upon impact. These devices have been extensively characterized for their ability to produce consistent and reproducible injuries (Basso et al., 1996; Beattie et al., 1997; Engesser-Cesar et al., 2005; Gruner, 1992; Jakeman et al., 2000; Scheff et al., 2003; Young, 2002). However, the question as to whether force or displacement is important in determining injury outcome is still not fully resolved (Stokes and Jakeman, 2002).

We now present experiments carried out using the IH impactor on adult mice to assess the influence of force and tissue displacement on injury outcome. The relationship between varying forces on tissue displacement and the effects on locomotor function and histological measures of cell and tissue damage were studied. These data show that there is a linear relationship between force and displacement and that both of these factors play a role in determining the anatomical and functional deficits after spinal cord contusion injury.

Materials and methods

Animals and surgeries

Adult female BALB/c mice (6–8 week old, 16–18 g; Charles River Canada, Saint-Constant, Quebec) were used. The protocol was adapted from that used for rats (Scheff et al., 2003) and approved by the McGill University Animal Care Committee following the guidelines of the Canadian Council on Animal Care. Briefly, the mice were anesthetized with ketamine:xylazine:acepromazine (50:5:1 mg/kg) and a partial laminectomy made using Mouse Laminectomy Forceps (Fine Science Tools [FST], Vancouver) at the 10th thoracic vertebral level. The mice were immobilized with modified serrated Adson forceps (FST) attached to the immediately adjacent vertebrae, and the contusion injury performed using the Infinite Horizons impactor device (Precision Scientific Instrumentation, Lexington, KY), as described previously (Engesser-Cesar et al., 2005; Scheff et al., 2003). A total of 39 mice were contused, plus 7 mice for the laminectomized controls. Three severities of injury were made based on the manufacturer-defined settings for mild, moderate, and severe injuries, at 30 (n = 9), 50 (n = 10), and 70 (n = 20) kdyn, respectively. Animals were sacrificed 28 days after injury.

Behavioral analysis

Mice (n = 5 for each of the contusion groups and n = 7 for laminectomy controls) were assessed for behavioral outcomes using the six-point Tarlov scale modified by Gale et al. (1985), which has been used to analyze locomotor recovery in spinal cord injured rodents (Faulkner et al., 2004; Fehlings and Tator, 1995; Kuhn and Wrathall, 1998). This scale analyzes gross aspects of hindlimb function, including voluntary movement, weight-bearing, and stepping. The mice were scored daily for the first week, and once a week thereafter for a total of 28 days. Briefly, the scoring scale used is: 0 = no movement and no weight-bearing; 1 = barely perceptible hindlimb movement and no weight-bearing; 2 = frequent hindlimb movement and no weight-bearing; 3 = occasional stepping and some weight-bearing; 4 = mild deficits in hindlimb function; 5 = normal stepping and weight-bearing. Each hindlimb was scored separately in an open-field by two individuals blind to the experimental conditions. The individual scores were pooled and averaged, for a maximum of 5 points for normal walking and weight-bearing. The data were analyzed using a two-way repeated-measures analysis of variance (RM-ANOVA) and comparisons between groups were made using a post hoc Tukey test, when appropriate. Only animals used for behavioral analysis were used for histological analysis.

Histology

Twenty-eight days after injury, mice (n = 4 for the mild and severe^{LD} groups, n = 5 for the moderate and severe^{HD} groups, and n = 7 laminectomy controls) were anesthetized and sacrificed by transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer. A 5 mm length of the spinal cord was removed, post-fixed for 1 h, and cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer. Serial cryostat sections (20 μm) were obtained for histological analyses and immunostained for: Mac-1, an integrin upregulated on activated macrophages/microglia; Hematoxylin and Eosin (H&E), used to examine overall tissue pathology and ventral motoneuron loss; and Luxol fast blue (LFB), used to identify areas of demyelination and length of the lesion. The protocols used were similar to those we have previously reported (De et al., 2003; Kalyvas and David, 2004; Ousman and David, 2000).

Quantification

Images were captured with a Retiga 1300 C digital camera (QImaging Corp., Burnaby, British Columbia) and all sections were analyzed with the BioQuant Nova Prime image analysis system (BioQuant Image Analysis Corp., Nashville) using a Zeiss AxioSkop II (Carl Zeiss Canada Ltd., Toronto) light microscope. The rostro-caudal extent of ventral motoneuron loss was measured from the first H&E stained tissue section lacking motoneurons to the last section before the cells appear. Macrophage activation was measured using the threshold feature where the percent of Mac-1 immunoreactivity was calculated for the total area for sections every 360 μm rostral and caudal to the epicenter of injury. The extent of myelin loss in contused spinal cord was calculated as a proportion of the area of myelin staining in laminectomized controls, while the rostro-caudal length of the lesion was measured by assessing the rostro-caudal length of myelin loss and was calculated in a manner similar