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Research Paper

A controlled spinal cord contusion for the rhesus macaque monkey

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ABSTRACT

Most in vivo spinal cord injury (SCI) experimental models use rodents. Due to the anatomical and functional differences between rodents and humans, reliable large animal models, such as non-human primates, of SCI are critically needed to facilitate translation of laboratory discoveries to clinical applications. Here we report the establishment of a controlled spinal contusion model that produces severity-dependent functional and histological deficits in non-human primates. Six adult male rhesus macaque monkeys underwent mild to moderate contusive SCI using 1.0 and 1.5 mm tissue displacement injuries at T9 or sham laminectomy (n = 2/group). Multiple assessments including motor-evoked potential (MEP), somatosensory-evoked potential (SSEP), MR imaging, and monkey hindlimb score (MHS) were performed. Monkeys were sacrificed at 6 months post-injury, and the lesion area was examined for cavitation, axons, myelin, and astrocytic responses. The MHS demonstrated that both the 1.0 and 1.5 mm displacement injuries created discriminative neurological deficits which were severity-dependent. The MEP response rate was depressed after a 1.0 mm injury and was abolished after a 1.5 mm injury. The SSEP response rate was slightly decreased following both the 1.0 and 1.5 mm SCI. MRI imaging demonstrated an increase in T2 signal at the lesion site at 3 and 6 months, and diffusion tensor imaging (DTI) tractography showed interrupted fiber tracts at the lesion site at 4 h and at 6 months post-SCI. Histologically, severity-dependent spinal cord atrophy, axonal degeneration, and myelin loss were found after both injury severities. Notably, strong astrocytic gliosis was not observed at the lesion penumbra in the monkey. In summary, we describe the development of a clinically-relevant contusive SCI model that produces severity-dependent anatomical and functional deficits in non-human primates. Such a model may advance the translation of novel SCI repair strategies to the clinic.

1. Introduction

Rodent models are used predominantly in spinal cord injury (SCI) research due to their ready availability and easy maintenance. Results obtained from rodent studies are useful but confirmation of these results in large animal models, such as in non-human primates, may facilitate transferring promising novel strategies to the clinic. This is because significant anatomical, functional, molecular, and pathological differences exist between the rodent and human spinal cords. For example, the corticospinal tracts (CST) are located in the dorsal funiculus of the spinal cord in rodents but in the posterolateral funiculus in primates and humans. CST damage causes mild, transient locomotor dysfunction in rodents but creates severe and permanent deficits in non-human primates (Anderson et al., 2009; Courtine et al., 2007; Hurd et al., 2013) similar to those seen in humans (Sterr et al., 2010).

Contusion SCI has been generated in rodents and large animals to study injury mechanisms and novel therapies (Deng et al., 2006, 2011; Iwanami et al., 2005; Kwon et al., 2015; Liu et al., 2006; Navarro et al., 2012; Sandler and Tator, 1976; Usvald et al., 2010). The Allen weight drop method, in which an object of known weight is dropped from a predetermined height, has been used to create a contusion SCI in monkeys (Albin et al., 1968; Crowe et al., 1997). The Allen method is simple

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and inexpensive but creates variable injuries due to respiratory movements that alter body position and angle of impact (Allen, 1911).

Current SCI models in rodents use clamps attached to the spinous processes that suspend the thoracic cage and thereby avoid chest compression (Kim et al., 2009; Wrathall et al., 1985). In the New York University (NYU)/MASCIS impactor device, vertebral motion was monitored at impact to ensure the reliability and reproducibility of the SCI (Zhang et al., 2008). Previously, we introduced the Louisville Injury System Apparatus (LISA) device in which vertebral stabilization was maintained by clamping facets of the spine bilaterally at the level of injury to avoid fracturing the fragile spinous processes (Blackmore et al., 2012; Zhang et al., 2008).

Several assessments have been developed to measure locomotion in untrained monkeys following SCI. Tarlov scores have been modified to assess hindlimb function; however, the reliability of this test in uncooperative monkeys is problematic (Babu et al., 2007). Alternatively, spontaneous activity in a cage can be recorded to assess motor function. Forelimb activity plays a major role in the movement of the monkey making it difficult to measure hindlimb function in the cage. Hindlimb functional assessment in the monkey has also been performed by unrestrained treadmill walking (Levi et al., 2002; Rosenzweig et al., 2010) and the restrained ball kicking test (Piedras et al., 2011). For public safety, function evaluation using enclosures with no restraint might be less stressful and more natural (Nout et al., 2012; Rosenzweig et al., 2010).

In the present study, we report the development of a novel controlled spinal contusion model for the adult monkey based on the previously reported LISA model for rodents. A modification of the LISA spine stabilization method was also used to stabilize the spine at the lesion level. This model, also called the LISA-Large (LISA-L) model, can create pre-determined tissue displacement of the dorsal spinal cord and, therefore, induce contusive SCIs at different severities. Using this model, we determined whether mild (1.0 mm) and moderate (1.5 mm) cord displacements in the monkey induced severity-dependent functional and histological differences. An array of electrophysiological, magnetic resonance (MR) imaging, behavioral, and histological assessments were chosen to determine whether different injury severities induced severity-dependent functional and anatomical deficits following SCI. We also developed a novel 12-point monkey locomotor scale (MLS) to assess hindlimb locomotor function of the monkey while walking within a 4.5 meter corridor. The major advantage of this measure is that the forelimb hanging and swinging of the monkey are restricted within the corridor.

2. Materials and methods

2.1. Animals

Six adult male rhesus monkeys (*Macaca mulatta*) (5.2 to 5.6 kg) with ages ranging from 3.5 to 4.2 years were used. They were divided into 3 groups (n = 2/group): sham operation, 1.0 mm displacement injury, and 1.5 mm displacement injury. All monkeys were sacrificed at 6 months post-injury. All monkey surgeries, postoperative care, and behavior studies were conducted at the Department of Laboratory Animal Sciences at Shanghai Jiao Tong University School of Medicine. All surgical interventions, treatments, and postoperative animal care were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, USA, 1996). The animal use protocol entitled "Spinal Cord Injury Research in Primates" was approved by the Animal Experiment Ethics Committee of the Shanghai Jiao Tong University School of Medicine (Approval number: 2013074), and all authors who were involved in the study were included in the above protocol. In this protocol, animal distress and conditions requiring euthanasia were addressed. Pain was minimized by the administration of Tramadol (Grunenthal Group, Aachen, Germany) (20 mg/kg) intramuscularly to monkeys on the first day post-surgery followed by 10 mg/kg Tramadol intramuscularly on the second and third day post-surgery. The number of animals used was minimized.

2.2. Spine stabilization and spinal cord exposure

Monkeys were anesthetized with intramuscular injection of ketamine (10–15 mg/kg). If they responded to noxious stimulus 10 min after induction, the anesthetic was supplemented by xylazine intramuscularly (2.0 mg/kg). Hair was shaved over the mid-thoracic spine, and the skin was scrubbed with iodine and alcohol. A 9–10 cm longitudinal midline skin incision was made to expose the T8–11 vertebrae, and paraspinal muscles were separated subperiosteally (Fig. 1A). The monkey was placed on the spine stabilizing device custom-designed to fix the vertebra and avoid motion of the spine during impact. A pair of adjustable T columns encircled the animal. Stainless steel arms connected to the spinal column were anchored to the T9 facets bilaterally. The chest was suspended using the vertebral stabilizing device to allow unrestricted respiration. A laminectomy of T9, exposing the T10–11 segments of the spinal cord, was performed using a high-speed drill (3 mm diameter fluted ball tip, Medtronic, Memphis, TN), and the ligamentum flavum was removed (Fig. 1B). The dura remained intact. The monkey was then transferred to the Louisville Injury System Apparatus-Large (LISA-L) impact device (Fig. 1C, D) with a laser sensor to determine precisely the distance of impact displacement. A detailed description of the LISA apparatus to produce a SCI in rodents has been described (Zhang et al., 2008). This apparatus has been modified for use in the monkey.

2.3. Producing a spinal cord contusion injury using the LISA-L Impactor

Mild to moderate SCIs were produced to avoid problems, e.g. sphincter disturbances and irreversible paralysis, in caring for paraplegic monkeys caused by severe SCI (Anderson and Stokes, 1992; Stokes, 1992; Zhang et al., 2008). From our earlier experience with rodent SCI models (Zhang et al., 2008), we anticipated that a 1.0–1.5 m/s injury at 1.0 mm and 1.5 mm displacement at T9 should deform the diameter of the spinal cord by 20–30%, which are equivalent to a mild and moderate SCI, respectively. Peak velocity of the plunger of 1.32 ± 0.05 m/s was produced using 30-psi compressed air. Contact duration of the plunger against the spinal cord was set at 0.25 ± 0.05 s. A 3.2 mm diameter plunger tip was used to produce a contusive SCI at the T9 vertebral level. After the injury, the spine stabilizer was detached, and muscles and skin were closed in layers. Animals were placed on a warm blanket in the recovery room until awake and administered fluid and food 1 h after recovery from anesthesia.

2.4. Monkey hindlimb score (MHS)

All monkeys were assessed for hindlimb function (n = 2/group) up to 4 months post-injury. The score of each monkey's hindlimb was considered as independent numbers so that the sample size was considered as n = 4/group. To assess hindlimb function, we developed a 12 point monkey hindlimb score (MHS) system that consisted of a hindlimb locomotor score (0–8 points) and a digital function score (0–4 points) (Table 1). The hindlimb locomotor score was assessed within a transparent corridor with dimensions of 4.5 m long, 0.35 m wide, and 0.75 m high. The corridor, with an arched ceiling, was fabricated using 10 mm thick Plexiglas sheets (Fig. 2). The dimensions of the corridor were determined by the size of the monkey (Hartman et al., 1933). The height of the corridor allowed monkeys to stand upright, and the width allowed them to turn. The corridor consisted of three retractable segments on wheels with two moveable steel grates between the two ends. Full expansion of the corridor (4.5 m long) was used during gait evaluation. The monkey entered the corridor through the door gate (green, Fig. 2A), and their gait was observed for 4–8 min. Food stimulus