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A simple, inexpensive and easily reproducible model of spinal cord injury in mice: Morphological and functional assessment

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ABSTRACT

Spinal cord injury (SCI) causes motor and sensory deficits that impair functional performance, and significantly impacts life expectancy and quality. Animal models provide a good opportunity to test therapeutic strategies *in vivo*. C57BL/6 mice were subjected to laminectomy at T9 and compression with a vascular clip (30 g force, 1 min). Two groups were analyzed: injured group (SCI, $n = 33$) and laminectomy only (Sham, $n = 15$). Locomotor behavior (Basso mouse scale—BMS and global mobility) was assessed weekly. Morphological analyses were performed by LM and EM. The Sham group did not show any morphofunctional alteration. All SCI animals showed flaccid paralysis 24 h after injury, with subsequent improvement. The BMS score of the SCI group improved until the intermediate phase (2.037 ± 1.198); the Sham animals maintained the highest BMS score (8.981 ± 0.056), $p < 0.001$ during the entire time. The locomotor speed was slower in the SCI animals (5.581 ± 0.871) than in the Sham animals (15.80 ± 1.166), $p < 0.001$. Morphological analysis of the SCI group showed, in the acute phase, edema, hemorrhage, multiple cavities, fiber degeneration, cell death and demyelination. In the chronic phase we observed glial scarring, neuron death, and remyelination of spared axons by oligodendrocytes and Schwann cells. In conclusion, we established a simple, reliable, and inexpensive clip compression model in mice, with functional and morphological reproducibility and good validity. The availability of producing reliable injuries with appropriate outcome measures represents great potential for studies involving cellular mechanisms of primary injury and repair after traumatic SCI.

1. Introduction

Animal models provide an opportunity to test therapeutic strategies *in vivo*, and are an important step on the way to clinical application. In recent years, spinal injury models have become more sophisticated in their reproducibility, behavioral analysis and comprehension of various sensory, motor and autonomic consequences (Reier, 2004). Experimental SCI models can maintain the continuity of the spinal cord, as in the contusion (e.g., weight-fall) or compression models, or not, as in the partial- or complete-transection models. In humans, the majority of acute SCI traumatic injuries is not followed by complete transection, but there is a combination of contusion, compression and possibly partial transection (Kakulas, 1984). Post-mortem examination of patients, classified according to criteria established by the American Spinal Cord Association (ASIA) as SCI functionally "complete" (grade A), i.e., no sensory or motor function below the level of injury, shows a small peripheral rim of spared axons (Hayes and Kakulas, 1997). A similar rim is also present in animal contusion and compression models (Bresnahan et al., 1987; Basso et al., 1996; Kamencic et al., 2001). The extradural clip compression injury model, as introduced by Rivlin and Tator (1978), simulates the clinical situation seen in the majority of human cases, and is less expensive than contusion models (Behrmann et al., 1992; Gruner, 1992; Scheff et al., 2003). However, the model selected must be appropriate to answer the questions raised by the researchers (Schwab et al., 2006).

Several models of SCI have been developed in small animals, mainly in rats. Genetically modified mice are widely used nowadays to study pathological events (Zheng et al., 2006; Saadoun et al., 2008), which would not have been possible in wild animals. Therefore, mouse models of SCI offer an advantage over other animal models, given the ease of testing the relationship between specific genetic mutations and the mechanisms that promote or prevent recovery (Basso et al., 2006).

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SCI causes motor and sensory deficits that impair functional performance, and has a significant impact on life expectancy and quality (Sekhon and Fehlings, 2001; Priebe et al., 2007). Although the survival rate has increased in recent years (to above 95%), these patients are subject to a lifelong handicap (Schwab et al., 2006). Nowadays, traumatic SCI and its devastating consequences represent one of the greatest challenges for those who work with nervous system degeneration and regeneration. Primary events after traumatic injury of CNS tracts are described as cell death (neurons, glia and endothelial cells) in the epicenter of the lesion, and stretching of axons, which can cause membrane damage, tract rupture and demyelination (Shi and Pryor, 2002). Secondary events, such as hemorrhage, hypoxia, edema, increase of free intracellular calcium, calpain activation, strong inflammatory response, astrogliosis, apoptosis, necrosis and excytotoxicity are triggered, and as a consequence progressively enlarge the primary area of lesion, in the rostral and caudal directions (Bareyre and Schwab, 2003; Park et al., 2004; Hagg and Oudega, 2006). Ascending and descending preserved tracts can, therefore, suffer further demyelination, mainly due to death or damage to oligodendrocytes, aggravating functional loss (Reier, 2004; Schwab et al., 2006).

In this study, we describe an effective experimental mice model that produces consistent SCI with little variability, and leads to reproducible histological and locomotor outcomes. We chose to use a compression model using a vascular clip with a known force of pressure to cause acute traumatic SCI; this injury simulates the clinical situation that is commonly observed in humans, and allows studies of functional spontaneous recovery. In this study, all the SCI animals showed complete hindlimb paralysis, with important functional deficits and histological alterations compatible with a moderately severe SCI.

2. Materials and methods

Our experimental study was approved by the Commission of Animal Care of the "Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro," under Protocol number DHEICB003.

2.1. Surgical procedure

Young adult female C57BL/6 mice were anesthetized by i.p. injection of a solution containing xylazine (15 mg/kg; Bayer, Brazil) and ketamine (100 mg/kg; Brazil), and then positioned on a cork platform. The skin was incised along the midline of the back, and the paravertebral muscles of the thoracic-level (T8–T10) vertebrae were dissected out. Laminectomy was performed at the T9 level under visual guidance, using an operating microscope (F104, OPTO, Brazil). One minute extradural compression with a vascular clip (with 30 g force, Kent Scientific Corporation, INS 14120, USA) was performed around the exposed spinal cord, in order to cause an acute-compression injury. Muscles and skin were sutured in layers, and an antibacterial spray was applied topically. Immediately after surgery, the animals were given a subcutaneous saline injection (1–2 mL) and prophylactic Baytril (2.5 mg/kg/d, s.c., Bayer, Brazil; Keirstead et al., 2005). They were left to recover on a warm pad until thermoregulation and an alert state was reestablished. They were then returned to their home cages, with free access to food and water. Animals received manual bladder expression twice daily until the return of bladder function; they also received appropriate veterinary care when needed. Two groups were analyzed: the injured group (SCI, $n = 33$) and the laminectomy group (Sham, $n = 15$).

2.2. Behavioral testing

We performed three types of tests: global mobility, locomotor performance and footprint. Prior to injury, all mice were acclimatised and scored for each test used. Global mobility test was developed to detect locomotor improvement after SCI throughout the analyzed time. The animals were evaluated by videotape with a WebCam (5 frames per second, for 1 min, KMEX, USA) using the free K3CCD software. Quantification was performed by means of the JAVA software (Jandel Video Analysis Software, Jandel Scientific, USA; Kunkel-Bagden et al., 1993). The animals' locomotor performance was analyzed using the Basso mouse scale open-field score (BMS, Basso et al., 2006) because it is a valid locomotor rating scale for mice. The evaluations were made by two blind observers for all analyzed groups. Briefly, the BMS is a 9-point scale that provides a gross indication of locomotor ability and determines the phases of locomotor recovery and features of locomotion. Early phase of recovery shows the resolution of paralysis and/or paresis progressed from no ankle movement to larger ankle movement and this is related to a score of 0–2. Plantar placing and the development of stepping occur in the intermediate phase of recovery, which requires score 3–4. In the late phase of recovery, paw position during stance, hindlimb–forelimb coordination and trunk stability are analyzed (score 5–8). Score 9 indicates the normal locomotor mobility, with trunk stability and refined performance.

The score for each hindlimb was recorded as the average per animal (left and right hindlimb) to obtain one BMS score per mouse, and then the mean of the group was calculated. For these tests, the animals were evaluated before injury, 24 h after surgery, and then weekly up to 8 weeks post-injury. Qualitative analysis of the forelimb and hindlimb step was made using the footprint test before surgery, and at 56 days after SCI. The forelimb and hindlimb plantar surfaces were inked with non-toxic red and blue dyes, respectively, and the mice were allowed to walk on white paper towards a dark tunnel (Ma et al., 2001).

2.3. Histology

After 8 weeks, the animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and perfused intracardiacally with a solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Spinal cords were dissected in three segments (at the epicenter of the lesion, and rostrally and caudally to it) and processed for light microscopy (LM) and electron microscopy (EM). Some animals ($n = 16$) were killed 7 days after injury and processed for LM ($n = 11$) and EM ($n = 5$), to analyze acute events after SCI. The other animals ($n = 17$) were killed 56 days after surgery and processed in the same way (LM - $n = 12$ and EM - $n = 5$). Sham animals were killed just at 56 days after surgery ($n = 15$). For LM, the spinal cord segments were embedded in OCT (Tissue Tek), and 10-μm-thick serial cross-sections were obtained with a cryostat (Leica CM 1850, Germany; $n = 5$ for Sham group, $n = 6$ in SCI acute group and $n = 8$ in SCI chronicle group) and collected on gelatin-coated glass slides. Longitudinal sections were used for qualitative analyzes ($n = 5$ for the three group analyzed). Sections were stained with Luxol Fast Blue (LFB), hematoxylin and eosin and Kluver–Barrera (LFB plus Nissl; Kluver and Barrera, 1953) to analyze white matter, injury site and neuron damage, respectively. Sections were observed under a Zeiss Axioscop 2 Plus Microscope and photographed with a Zeiss Axiocam MRC camera, using the Axiovision program, version 4.5 (Zeiss) for image acquisition. For EM ($n = 5$ for each group), the spinal cord segments were post-fixed by immersion in cacodylate-buffered 1% osmium tetroxide with 0.8% potassium ferrocyanide for 7 h, at room temperature. Tissue was washed in 0.1 M phosphate buffer