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Laboratory Investigation

A Mouse Model of Photochemically Induced Spinal Cord Injury

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Objective : A mouse model of spinal cord injury (SCI) could further increase our basic understanding of the mechanisms involved in injury and repair of the nervous system. The purpose of this study was to investigate whether methods used to produce and evaluate photochemical graded ischemic SCI in rats, could be successfully adapted to mice, in a reliable and reproducible manner.
Methods : Thirty female imprinting control region mice (weighting 25-30 g, 8 weeks of age) were used in this study. Following intraperitoneal injection of Rose bengal, the translucent dorsal surface of the T8-T9 vertebral laminae of the mice were illuminated with a fiber optic bundle of a cold light source. The mice were divided into three groups; Group 1 (20 mg/kg Rose bengal, 5 minutes illumination), Group 2 (20 mg/kg Rose bengal, 10 minutes illumination), and Group 3 (40 mg/kg Rose bengal, 10 minutes illumination). The locomotor function, according to the Basso-Beattie-Bresnahan scale, was assessed at three days after the injury and then once per week for four weeks. The animals were sacrificed at 28 days after the injury, and the histopathology of the lesions was assessed.
Results : The mice in group 1 had no hindlimb movement until seven days after the injury. Most mice had later recovery with movement in more than two joints at 28 days after injury. There was limited recovery of one joint, with only slight movement, for the mice in groups 2 and 3. The histopathology showed that the mice in group 1 had a cystic cavity involving the dorsal and partial involvement of the dorsolateral funiculi. A larger cavity, involving the dorsal, dorsolateral funiculi and the gray matter of the dorsal and ventral horns was found in group 2. In group 3, most of the spinal cord was destroyed and only a thin rim of tissue remained.
Conclusion : The results of this study show that the photochemical graded ischemic SCI model, described in rats, can be successfully adapted to mice, in a reliable and reproducible manner. The functional deficits are correlated an increase in the irradiation time and, therefore, to the severity of the injury. The photothrombotic model of SCI, in mice with 20 mg/kg Rose bengal for 5 minutes illumination, provides an effective model that could be used in future research. This photochemical model can be used for investigating secondary responses associated with traumatic SCI.

KEY WORDS : Photochemical · Spinal cord Injury · Mouse.

INTRODUCTION

Spinal cord injury (SCI) typically leads to permanent neurological deficits. Currently, there are no therapeutic agents available that have proven efficacy for restoring motor function after SCI in humans. Experimental models of SCI have improved our basic knowledge of the mechanisms associated with secondary injury of nervous tissue after damage[12]. The weight drop technique remains the most widely used method of inducing SCI experimentally. This technique offers the advantage of being clinically relevant, because most human injuries involve tissue damage due to rapid movement of the vertebral column with the impact of bone against the spinal cord.

A different approach, the photochemical model of SCI, has been widely used in neurotraumatic research over the past two decades. This method can be used to produce reliable graded lesions of predetermined severity with minimal variability in rats[13]. This model is based on the photochemical reaction between photosensitive dyes and light that generate singlet molecular oxygen. Rose bengal (RB, Sigma-Aldrich, MO, USA) is the most effective

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generator of singlet oxygen; it reacts with structural proteins and lipids to initiate direct peroxidation reactions within endothelial membranes[8]. When RB is absorbed into the blood flow and focal illumination activates the local dye, free radical production results. This causes vascular endothelial damage and platelet aggregation with subsequent vascular occlusion, edema and tissue necrosis, simulating the secondary response seen after traumatic SCI[4,5,9,14]. In the early stages of tissue infarction, occlusive platelet thrombi, within the surface and parenchymal vessels of the cord, may directly suppress spinal cord blood flow[9].

Currently, mice are frequently used to study the genetics of disease using transgenic and knock-out strategies. Mice models of SCI could further advance our knowledge on the mechanisms of injury and repair of nervous system. It is well known that important differences exist between mice and rats in their response to central nervous system injury[11,13]. Therefore, to evaluate the therapeutic strategies in the fields of pharmacological, cellular and genetic approaches to neurotrauma, the development of a reliable and reproducible SCI model in mice is needed. The aim of this study was to adapt rat photochemical SCI model for use in mice by modifying the application route of the dye and illumination parameters.

MATERIALS AND METHODS

Animal preparation

Thirty female Imprinting Control Region (ICR) mice, weighing between 25 and 30 g (aged 8 weeks), were used in this study. Females were used in order to facilitate management of posttraumatic urinary dysfunction. The mice were kept in 12-h light/dark cycles, and allowed free access to food and water.

Surgical procedure

All surgical procedures and postoperative care were performed in accordance with the guidelines of the Animal Care and Use Committee. Spinal cord lesions were induced by photothrombosis of the cord microvessels according to a modified method described by Watson et al.[13] Each mouse was anesthetized with 2% isoflurane and maintained with 1% isoflurane in an oxygen/air mixture using a gas anesthesia mask in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). After obtaining a deep level of anesthesia, the mouse was placed in a prone position with a warmed surgical pad to preserve body temperature throughout the procedure. The rectal temperature was controlled during surgery at 37 ± 0.5°C with a homeothermic blanket (Harvard Apparatus). A midline incision of the skin was made; the vertebral muscles were dissected bilaterally exposing the dorsal laminae and spinous processes of the T8-T10 vertebrae. Then 0.1 ml RB was administered intraperitoneally. Five minutes later, the irradiation beam of a cold light source (Zeiss KL1500 LCD, Jena, Germany) with a 4.5 mm aperture, was focused over the translucent dorsal surface of the T8-T9 vertebral laminae. After illumination, the back muscles and skin were sutured. The animals were then returned to their individual cages, and kept heated under an infrared lamp until full recovery from anesthesia. Antibiotic coverage was provided for five days and the bladder was emptied twice a day by manual pressure until total recovery of function. All animals were divided into three groups depending on the desired injury grade : Group 1 (20 mg/kg RB, 5 minutes illumination, n = 10), Group 2 (20 mg/kg RB, 10 minutes illumination, n = 10), and Group 3 (40 mg/kg RB, 10 minutes illumination, n = 10).

Behavioral study

Behavioral recovery was assessed for four weeks after the SCI in an open field environment by the Basso-Beattie-Bresnahan (BBB) locomotor rating scale[6]. The BBB scale ranges from 0 (no observable hindlimb movement) to 17 (consistent plantar stepping with consistent weight support, consistent coordination; the predominant paw position is parallel to the body, flat toes and consistent stability in the locomotion). Scores from 0 to 7 indicate the return of isolated movements in the three joints (hip, knee, and ankle). Scores from 8 to 12 indicate the return of paw placement and coordinated movements with the forelimbs, whereas scores 13-17 show the return of the toe clearance during stepping, predominant paw position, trunk stability, and tail position.

Histopathological study

All the mice were anesthetized and perfused transcardially with 4% paraformaldehyde in PBS. The spinal cords were removed, fixed in the same solution overnight at 4°C before removed. The cords were blocked in cross or longitudinal section and processed for paraffin embedding. Representative sections were sliced into 4 μm-thickness sections and stained with hematoxylin-eosin (H&E) and cresyl violet. The immediately adjacent sections were processed simultaneously for immunohistochemistry (IHC) with antibodies to; GFAP [1:400, rabbit polyclonal (Millipore, Bedford, MA, USA)], MAP2 (1 : 400, mouse monoclonal, Millipore), NF-M/H [1 : 400, mouse monoclonal (Dako, Copenhagen, Denmark)] and CD68 (1 : 500, mouse monoclonal, Millipore). For the IHC, the tissue sections were collected on aminopropyltriethoxysilane (APTE)-coated slides and immunostained with the avidin-biotin conjugation method using a Sequenza Rack (Shandon, UK). Endogenous peroxidase activity was blocked by incubation in phosphate buffered saline (PBS, pH 7.4) containing 1.5% H_2O_2. Pretreatment of the tissues with heat-induced epitope retrieval was required for 3 min at 125°C in a pressure cooker with 10 mM citrate buffer, pH 6.0 for GFAP, MAP2, NF-M/H and CD68 antibody. The slides were incubated with each primary antibody overnight at 4°C. The streptavidin-horseradish peroxidase (Dako) detection system was then applied. Antibody diluent (Dako) was also applied as a negative control stain.

Statistical analysis

Values are presented as the mean ± standard error of the mean (SEM). Statistical comparisons among groups were made by repeated-measure ANOVA and the Mann-Whitney U test for the functional outcomes. Differences were considered significantly at a $p < 0.05$.

RESULTS

Functional outcome

With regard to the functional evolution, the mice in group I showed an initial mild-to-moderate functional impairment; the group 1 mice had better locomotor function than groups II and III until the end of the follow-up period. On the other hand, the mice in groups II and III initially had severe functional impairment reflected by a loss of about 70-90% of their mean gross motor function. From seven days after the SCI to the end of the experimental period, the mice in group I were significantly different from the mice in groups II and III (Fig. 1). The functional deficits were associated with the increase of illumination time and the amount of RB. The grade of the injury was correlated to the severity of the functional impairment.

Histopathological findings

The cord lesion was consistently made with equal size in width. However, the depth of the lesion was quite different among groups. The spinal cord sections from the mice in group I showed a small cavity affecting the dorsal and dorsolateral funiculi and extending to the central canal. The ventral horns, as well as the ventrolateral and ventral funiculi remained intact (Fig. 2A, C). The spinal cord sections from the mice of group II showed a larger cystic cavity affecting the dorsal and dorsolateral funiculi and the gray matter of the dorsal and ventral horns. These lesions were replaced by macrophages. Neurofilament protein (NF) was expressed in neurons and its processes in the non-lesional cord (Fig. 2B, D). In group I, NF positive neurons were observed in the remnant neuritic process, but in group II and III, large amounts of fragmented neuritis in the lesional boundary. The cystic lesions were filled with CD68 + histiocytes (data not shown).

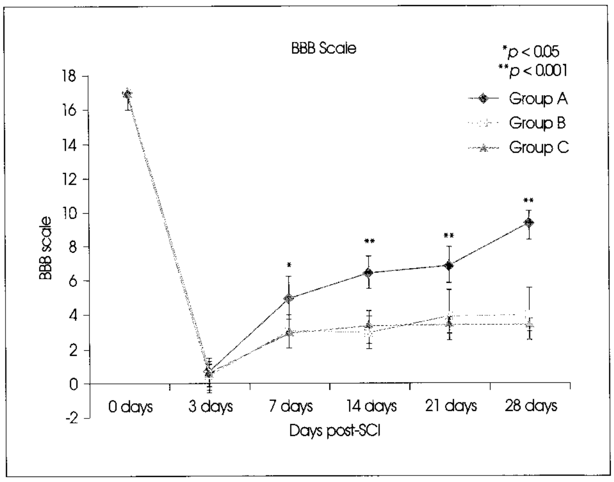

Fig. 1. Analysis of functional recovery.

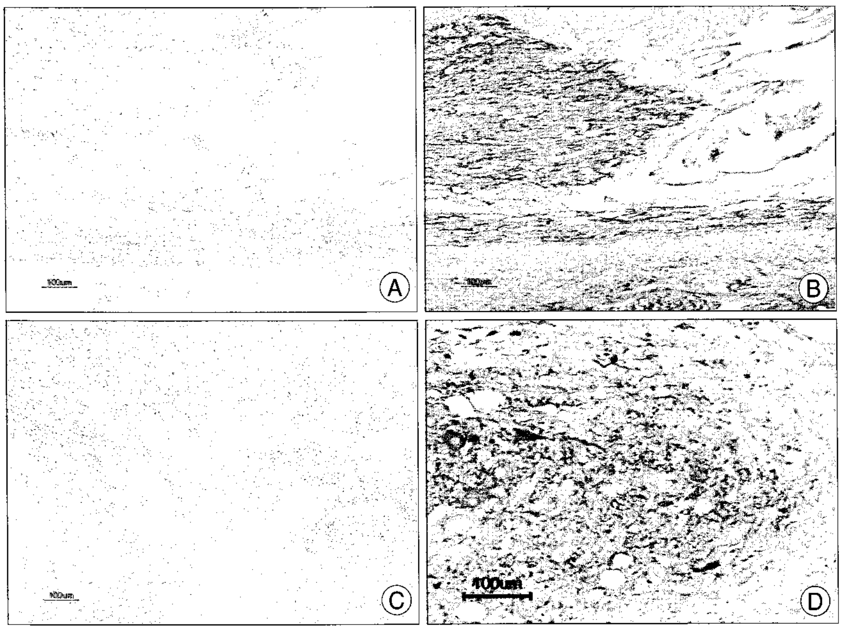

Fig. 2. Histopathologic findings of photothrombotic spinal cord injury model in mice. In group I (A and B) shows partial destruction and remnant axonal component in H&E staining and neurofilament protein (NF) immunohistochemistry. The group II and III models (C and D) show large cystic cavitary lesions filled with foamy histocytes (C, H&E staining) and abnormally fragmented neuritis (D, NF immunostaining). Each scale bar is 100 um.

DISCUSSION

The pathophysiology of acute SCI is complex and includes a two-step process of primary and secondary damage[7]. The primary injury refers to the structural damage caused by the initial mechanical trauma; this is followed by the spread of secondary tissue damage that expands from the injury "epicenter". Many of the pathological changes seen after a SCI are thus secondary to the initial impact and include edema, altered blood flow and changes in the microvascular permeability. Local vascular alterations and ischemia within the spinal cord are thought to be one of the most important aspects of the secondary injury. Therefore, the ischemic photochemical model of a SCI in mice represents a useful model that potentially can play a key role in neurotrauma research.

A variety of methods have been employed to produce SCI, including; complete or partial transections[3], crushing the cord with forceps or aneurysm clips[10], contusion injury from mechanical impact[1] and photochemical lesions using RB or erythrosis B[14]. Complete transection models, in which the spinal cord is fully transected, make it somewhat easier to evaluate the effectiveness of interventions with regard to both axonal regeneration and functional recovery[7]. However, functional recovery does not occur spontaneously in this model. In partial transection models, an attempt is made to cut tracts of the spinal cord selectively. This approach might allow for comparison of the regenerative response in a particular tract with its uninjured partner on the contralateral side. Most of the corticospinal tracts in rats descend in the ventral aspect of the dorsal column, just dorsally to the central canal. In dorsal hemisection models, the lesion transects the rubrospinal and corticospinal tracts bilaterally. Partial transection models are useful for the study of anatomic regeneration of axons despite the lack of applicability to the vast majority of blunt SCI. The weight drop technique, originally introduced by Allen in 1911[1], remains the most widely used method for experimentally induced SCI. In human SCI, even with complete paraplegia after blunt injury, the cord rarely is completely transected, but rather leaves some residual, normal-appearing cord parenchyma peripherally at the injury zone. The weight-drop contusion models produce a similar lesion, in which neuronal tissue remains intact along the peripheral rim. The rat model of contusive SCI has been shown to provide a reliable, reproducible, graded injury, and offers a easier method for establishing large-scale screening of potential therapeutic agents[2]. However, the surgical processing was complex, and sufficient experience with strict adherence to control of variability of the injury is necessary to produce a consistent and reliable injury. Nonetheless, it might be difficult to depend on contusive SCI models in mice due to their size and the potential difficulty in reproducing a consistent injury.

The photochemical damage of the spinal cord has histopathological similarities with contusion and compression experimental lesions. The photochemical lesion is generated principally as a consequence of microvascular occlusion[9]. The photochemical models in rats have shown that there is a prolonged secondary injury phase associated with photochemical lesions[11,13]. Acute responses to the endothelial damage include platelet aggregation to the point of vascular occlusion and vasogenic edema that is usually sufficient to occlude the deeper microvasculature by mechanical compression[11]. This compression effect extends in uniform fashion the zone of "induced" photochemical damage beyond the depth of the microvasculature that was directly damaged by the photochemistry[11,13]. We demonstrated that graded SCI can be induced by a photochemical lesion in mice. The mechanical injury rarely transects the cord completely in clinical SCI seen in patients after trauma. These results prove the efficiency of the photochemical approach to produce partially lesioned spinal cord. The graded behavioral and histopathological abnormalities can be produced depending on not only the time of illumination but also the concentration of the RB. A consistent progression of histopathological abnormalities could be established, reproducing experimentally controlled, graded ischemic SCI in mice. The locomotor outcomes showed a gradual degree of impairment related to the severity of the SCI. After five minutes of illumination, the infarct area included the dorsal and dorsolateral funiculi, excluding the ventrolateral and ventral funiculi; the infarct area of the entire cord thickness was seen after 10 minutes illumination. Despite the profound initial impairment in severely injured mice, the animals partially recovered hindlimb motor function during the first week after the injury. Mice with exposures to 5-minutes of illumination demonstrated a significant improvement of functional motor recovery at 1 week after the cord injury whereas the mice with 10-minute illumination injuries showed no improvement. The partial locomotor recovery could be explained by the preserved ventrolateral quadrant of the cord at the lesion site.

Therefore, the photochemical lesion provides a model system that not only it is able to induce reproducible, moderate lesions in the spinal cord of mice, but also it reliably spares peripheral regions of the cord. Since ischemia represents one of the main aspects of traumatic SCI, the photochemical model might be useful for investigating this component of traumatic SCI. This model also might be suitable for the study of the contribution of vascular injury to degeneration of spinal cord tissue. Another advantage of the photochemical model is that laminectomy is not required for inducing the lesion; the translucency of the vertebral lamina allows irradiation of the underlying vasculature[5,9,14]. Furthermore, the surgical procedure is noninvasive with regard to the spinal cord because it can be performed without laminectomy and cord manipulation. Therefore, animals rapidly recover from surgery with improved long term survival. In addition, the size and location of the infarction can be altered by changing the position of the light source and the duration/intensity of illumination, allowing focal ischemia in the different regions. Application of such an experimental approach would allow for spinal cord tracts in specific regions to be selectively damaged or left intact.

CONCLUSION

In summary, the results of the present study show that the photochemical graded ischemic SCI model, described in rats, can be successfully adapted to mice, in a reliable and reproducible manner. Our results demonstrate that functional and histological consequences can be made progressively more severe by increasing the illumination time and the concentration of RB. The ischemic photochemical model of SCI can be successfully adapted to mice using 20 mg/kg RB for 5 minutes of illumination. This experimental model can be used to investigate different therapeutic strategies for the promotion of neuroprotection and central regeneration in SCI.

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References

1. Allen AR : Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. A preliminary study. **JAMA** 57 : 878-880, 1911
2. Black P, Markowitz RS, Damjanov I, Finkelstein SD, Kushner H, Gillespie J, et al : Models of spinal cord injury : Part 3. Dynamic load technique. **Neurosurgery** 22 : 51-60, 1988
3. Bregman BS, Goldberger ME : Anatomical plasticity and sparing of function after spinal cord damage in neonatal cats. **Science** 217 : 553-555, 1982
4. Bunge MB, Holets VR, Bates ML, Clarke TS, Watson BD : Characterization of photochemically induced spinal cord injury in the rat by light and electron microscopy. **Exp Neurol** 127 : 76-93, 1994
5. Cameron T, Prado R, Watson BD, Gonzalez-Carvajal M, Holets VR : Photochemically induced cystic lesion in the rat spinal cord. I. Behavioral and morphological analysis. **Exp Neurol** 109 : 214-223, 1990
6. Dergham P, Ellezam B, Essagian C, Avedissian H, Lubell WD, Mckerracher L : Rho signaling pathway targeted to promote spinal cord repair. **J Neurosci** 22 : 6570-6577, 2002
7. Kwon BK, Oxland TR, Tetzlaff W : Animal models used in spinal cord regeneration research. **Spine (Phila Pa 1976)** 27 : 1504-1510, 2002
8. Pooler JP, Valenzeno DP : Dye-sensitized photodynamic inactivation of cells. **Med Phys** 8 : 614-628, 1981
9. Prado R, Dietrich WD, Watson BD, Ginsberg MD, Green BA : Photochemically induced graded spinal cord infarction. Behavioral, electrophysiological, and morphological correlates. **J Neurosurg** 67 : 745-753, 1987
10. Rivlin AS, Tator CH : Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. **Surg Neurol** 10 : 38-43, 1978
11. Steward O, Schauwecker PE, Guth L, Zhang Z, Fujiki M, Inman D, et al : Genetic approaches to neurotrauma research : opportunities and potential pitfalls of murine models. **Exp Neurol** 157 : 19-42, 1999
12. Tator CH, Fehlings MG : Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. **J Neurosurg** 75 : 15-26, 1991
13. Watson BD, Holets VR, Prado R, Bunge MB : Laser-driven photochemical induction of spinal cord injury in the rat : Methodology, histopathology, and applications. **NeuroProtocols** 3 : 3-15, 1993
14. Watson BD, Prado R, Dietrich WD, Ginsberg MD, Green BA : Photochemically induced spinal cord injury in the rat. **Brain Res** 367 : 296-300, 1986