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Research Article

Modeling of Post Traumatic Glial Scar after Controlled Cryodestruction of Rat Spinal Cord

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Abstract

Spinal cord injury (SCI) represents a relevant problem under both clinical e social points of view. Post traumatic glial scarring is recognized as one of the main factors which would affect negatively axons regrowth following SCI. As in the situation of SCI occurring in humans, presently available animal experimental model of SCI provoke a wide range of glial scarring reaction as a result of trauma, thus investigating the specific issue of post traumatic glial scarring would require an extremely high number of animal due to significant inter animals variability. We recently introduced a new model of experimental SCI in rats where a controlled unilateral cryo-induced lesion was created. Sprague-Dawley rats of adequate size were used, microsurgical unilateral exposure of lower thoracic spinal cord dura was performed, and cryolesion was induced via a controlled exposure to -20° temperature for one minute using liquid azote. The animals were sacrificed following 30 days of close observation during which they developed a consistent unilateral lower limb deficit. Microscopical examination of the injury site demonstrated spinal cord tissue vacuolization of rather consistent sites and surrounded by glial scarring very similar in extension in different animals. Preliminary results indicate that either produced lesions either glial scarring are rather consistent. This model may be of value in investigating the possible therapeutic role of pharmacological protocols for SCI.

Keywords: *Spinal Cord; Experimental Model; Cryoinjury; Glial Scar; Controlled Spinal Lesion*

Introduction

Spinal cord injury (SCI) represents a relevant medical-social problem since it affects mostly relatively young people with long life expectancy and a significantly reduced working capacity coupled with highly demanding care regime [1,2]. Extensive research of possible specific therapeutic solutions has not yet provided results of significant impact [3,4]. Therefore, it appears evident that reliable, easily reproducible experimental animal models are needed for testing possible therapeutic solutions. Rodent models of experimental SCI, either by compression-simulating impact or by contusion-simulating impact [5] in small animals are the most widely used experimental model for studying spinal trauma. Although they would reproduce the actual SCI as it occurs in humans, they, as in the clinical situation, appear to suffer from the shortcoming of being too difficult, if not impossible, to standardize. In particular, production of glial scar after these experimental traumatic injuries is highly variable, and this fact represents a real problem when therapeutic solutions should be evaluated

experimentally [6]. Considering that the posttraumatic glial scar either impedes or at least makes extremely difficult axonal regeneration across the lesion site in SCI, it appears consequential that potentially more affective ways for reliably reproducing controlled glial scars following experimental SCI should be investigated. We recently developed another technique for experimental SCI in rats using regional cryodestruction, which seems to produce standardized glial scarring [7], thus could be used as very reliable model of posttraumatic scarring in order to test potential therapeutic solutions. The technique is described below.

Materials and Methods

Male specific-pathogen-free (SPF) rats Wistar category form Animal breeding facility of BIBCh RAS, Pushchino (USU "Bio-model" IBCh RAS) weighting approximately 500g were used for this purpose. Experiments were approved by the institutional animal care and use committee (IACUC) of our Institution. The optimal injury location was performed at the end of the thoracic segment of the spinal cord, at Th13 level. This took into account minimal depth of surgical approach and anatomical characteristics of the spinal cord, specifically cauda equina branching and transverse diameter of the spinal cord. This infact represents the principal according to which we selected the proper site of cryoinjury. The animals were anesthetized with Florane. The Th13 spinous process was identified at the convergence of the aponeuroses of the erector spinae muscle. Unilateral hemilaminectomy was performed using 1 mm diamond burr. Integrity of exposed spinal dura mater was confirmed by the absence of cerebrospinal fluid (CSF) leak. Injury was produced by a copper cryo-conductor (Ø 0.8 mm) and cryo-destruction performed by local application of minus 20º controlled thermal injury for 1 minute (Figure 1).

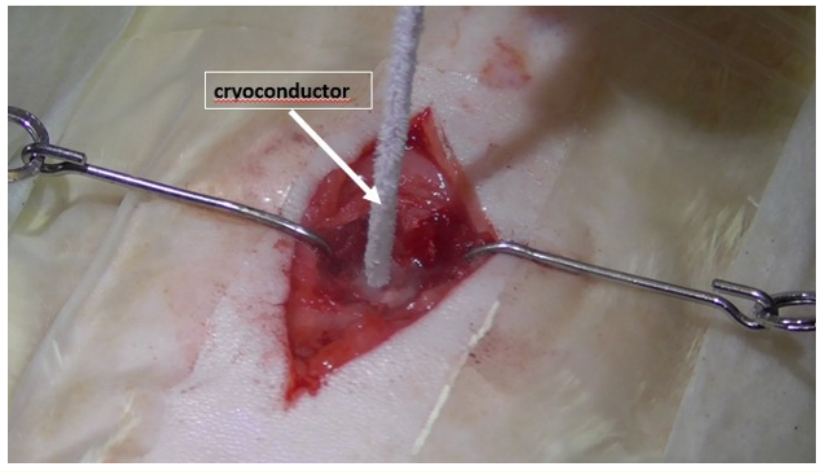

Figure 1: *View of the microprobe in place for thermal injury.*

Results

Following 30 days of careful monitoring, animals are sacrificed, three vertebral (T12-L1) segments are removed, and macroscopic (Figure 2) as well as microscopic (Figure 3) appearance of the lesioned area is inspected and compared with the contralateral non-traumatized area. Microscopically the lesions appeared to be standardized and glial scarring pattern was comparable between different animals (Figure 3).

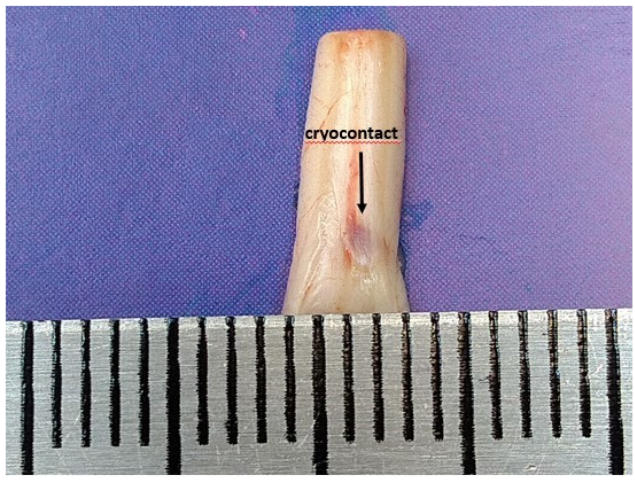

Figure 2: *Macroscopic view of the lesioned spinal cord.*

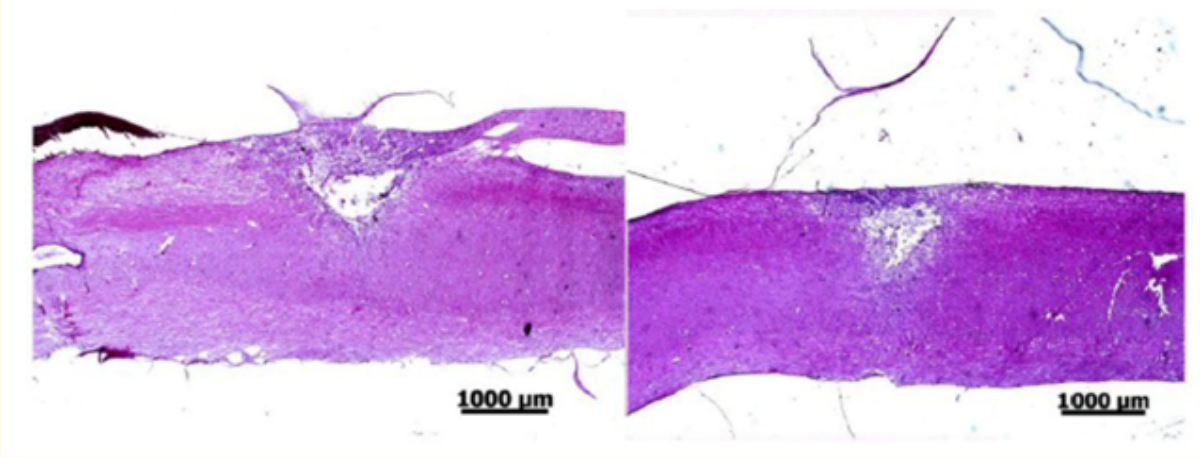

Figure 3: *Sagittal histological cut of two different animals. Note the actual reproducibility of the cryo-lesion.*

During the postoperative period, the animals retained the functions of defecation and urination. The clinical symptoms in animals subjected to cryo application were characterized by monoplegia and trophic changes in the distal extremities from the side of the injury.

Discussion

The idea of modeling glial scars by cryo-application on the spinal cord of rats was based on studies of the effects of cryo-destruction on glial tissues [8,9] and of cryo-analgesia [10,11]. Cryo-neurolysis-induced nerve trauma with temperatures ranging from - 20°C to -100°C causes morphological changes similar to those found with Wallerian degeneration after mechanical injury and is characterized

by a reversible degeneration of the axons distal to the injury site [11-15]. Likewise, a mechanical trauma, cryo-neurolysis destroys the endoneural channels as well as Schwann cells [16,17], thus causing growth of scar tissue which affects negatively the recovery of axons [18,19]. However, the non-affected basal membrane makes the process reversible, since it would serve as a bio-carcass to be used in the course of the regenerative recovery [20]. Only extremely low temperatures (-140°C) induce an immediate and irreversible necrosis due to destruction of the basal membrane [21]. Studies on trauma bio simulation by cryo-neurolysis were typically conducted on the isolated sciatic nerve (SCN) of rats and, less frequently, rabbits [22-24]. Either nitrogen oxide or carbon dioxide were commonly used as cryogens [25]. This technique is considered to be safe, since the temperature levels used for the experimental cooling of the tissues are not lower than the boiling points of the used gases (-88°C for nitrogen oxide and -79°C for carbon dioxide). There are, though less frequently, studies on the effect of cryo lesioning on the central nervous system (CNS), particularly in the spinal cord. As far as ascending fibers of dorsal group of rat spinal cord several investigations proved that the cellular matrix of nerve fibers had a potential for self-regeneration [26]. Highlighted the direct cell-mediated support to the axon growth provided by microglial components, macroglia and Schwann cells generated from the population of local stem cells, astrocytes and oligodendrocytes. In general cryoinjury is considered as a relatively safe method. It has no cumulative effect resulting from repeated use [27]. Cryo lesion is created in the present experimental study using a standardized and reproducible technique. Results of histological studies performed on the animals used for this investigation although preliminary ones appear to indicate that cryo application leads to highly reproducible, standardize spinal cord injuries. This would appear an extremely interesting observation since it might introduce a novel model of controlled SCI which would produce standardized injuries. This in turn would make it a very reliable model for investigating therapeutic measures for either preventing or effectively treating post-traumatic glial scars, which negatively affects and in fact impede axonal regeneration on the injury side.

Conclusion

Recent experimental model of spinal cord cryoinjury produces consistent post traumatic glial scars and can be useful for studying pathophysiology and possible therapeutic options specifically directed towards this relevant complications of spinal cord trauma.

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