Characterization of rat contusive spinal cord injury model and treatment with local medicated scaffold

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INTRODUCTION

Spinal cord injury is a devastating condition primarily caused by preventable accidents such as road traffic crashes, falls or violence. Up to 90% of spinal cord lesion are caused by trauma, though the proportion of non-traumatic spinal cord injury appears to be growing. Symptoms of spinal cord injury depend on the severity of the injury and its location in the spinal cord. Symptoms may include partial or complete loss of sensory function or motor control at arms, legs and/or body. The most severe spinal cord injury also involves bowel or bladder control, breathing, heart rate and blood pressure regulation. Most people with spinal cord injury experience chronic pain. There are no treatments for the acute phase of the spinal cord injury, being the steroid therapy almost virtually abandoned.

The general aim of this three-year project is to develop novel therapeutic solutions for the acute phase of the spinal cord injury. The specific aims of the first year are:

1. The morphological and functional characterization of the model of contusive spinal cord injury in the rat at different post-injury times;
2. The study of the synaptic plasticity in the spinal cord and brain motor circuits after spinal cord lesion at different post-injury times;
3. Pilot experiments aimed to the implantation of medicated PLLA scaffold in lesioned animals are also presented.

METHODS

CD-Sprague Dawley (Charles River, Italy) female rats, 225-300 g body weight, were used in this study. Spinal cord injury was performed using a 10 mm diameter impactor (Leica Biotecsystems, Germany) using a force of 1 N at 0.075 m/s and 0 s of stance time, the depth of impact was 2 mm in order to reach the ventral horns of gray matter. For functional characterization we used 30 animals per group, for evaluation of lesion volume area, synaptic plasticity analysis and implantation of medical scaffold were used 5 animals per group. An equal number of sham-operated animals (laminectomy, only) were used as control.

Evaluation of hind limb functional locomotor loss was assessed with Basso-Bea and Bresnahan) and gait analysis was performed with CatWalk (Noldus, The Netherlands) automated system. For the definition of lesion area was then determined for each reconstructed section with ImageJ (NIH) as number of pixels occupying the lesion site.

mRNA real-time PCR was performed to study gene expression. The relative expression of all studied genes was calculated through the 2^(-ΔΔCt) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value. Graphs show mean ±SEM, statistical analysis was performed by 2-way ANOVA (*) or T-test (8). Significant differences have been indicated.

1. The morphological and functional characterization of the model of contusive spinal cord injury in the rat at different post-injury times

A. Body Weight

B. Clinical Score

C. BBB Score

D. Print Area

E. Max Contact Area

F. BOS

G. Slide Length

H. Step Cycle

Panel A. Body weight gain after surgery procedure in experimental animal, expressed as % changes respect to pre-surgery, lesioned animals show a remarkable decrease in body weight after contusion, which is partially recovered over the time.
Panel B. The clinical score evaluated behavior, wound closure, infections and tissue index (left). Lesion area was calculated through the 2(-ΔΔCt) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value.
Panel C. Lesion area was calculated through the 2^(-ΔΔCt) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value.
Panel D. Cluster analysis of gene expression for the array "inhibitory whole spinal cord lesion at different post-injury times". Legend: Sham operated vs injured spinal cord. Lesioned animals show a massive gene expression regulation compared to control group. Lesioned animals at 8 days post surgery showed a higher clinical score in the first two weeks after surgery.

2. The study of the synaptic plasticity in the spinal cord and brain motor circuits after spinal cord lesion at different post-injury times

A. Longitudinal 3D reconstruction of lesioned spinal cord at 8 days (E, D) and 45 (E, F), T3 days post injury, lesion area was calculated through the 2^(-ΔΔCt) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value.

B. Longitudinal 3D reconstruction of lesioned spinal cord at 8 days (E, D) and 45 (E, F), T3 days post injury, lesion area was calculated through the 2^(-ΔΔCt) method. The control experimental group was used as reference mRNA, so that values are represented as x-fold of control mean value.

3. Implantation of medicated scaffold in lesioned animals; preliminary data (patent application presented)

A. TNFa

B. IL-1b

Panel A. Gene expression analysis of TNFa in rostral and caudal segments of lesioned spinal cord of animals implanted with treated or untreated scaffolds. Expression of TNFa in rostral segment was increased by treated scaffold at both time points, while in caudal segment TNFa was decreased at 8 days post lesion.
Panel B. Gene expression of IL-1b in rostral and caudal segment of lesioned spinal cords. IL-1b expression was unchanged in rostral segment while in caudal segment treated scaffold decrease expression of IL-1b at both 24 hours and 8 days post injury.

Concluding remarks and future perspective

Our animal model shows all clinical characteristics of spinal cord lesion (neurological bladder, clinical recovery and hind limb paralysis) and present a high reproducibility and reliability in clinical symptoms and expressed as ratio compared to total area. The biological response to the SCI is divided into three phases: acute (a few seconds or minutes after the injury), secondary (from a few minutes to a few weeks to several months to a few years after the injury). In the acute phase vascular events are prevalent such as edema and serious alterations of the chemical microenvironment (tonic homeostasis, accumulation of neurotransmitters, plasma membrane compromise, etc.). Many of these events are also present in the secondary phase, including oxidative stress, an and immunological inflammatory reaction, the initiation of astroglial scaring, and demyelination leading to the electrophysiological collapse. In the chronic phase the demyelination, the astroglial reaction and the central cavitation continues, while the regeneration attempts, for example the sprouting by some neurons, determines alterations in the anatomy and physiology. The aim of this three year project is to develop implantable scaffolds to prevent/limit secondary neurodegeneration, targeting inflammation and remyelination. The first year activities provided the model, related variability, reliable end-point for efficacy studies, and resolution of all technical problems related to the scaffolds implantation (PLLA elexciron scaffold mediated with two drugs, patent application presented). Local treatment with medicated scaffold implanted immediately after the spinal cord lesion reduces inflammation in the segment below the lesion site during the acute phase of inflammation.