The Effect of ChABC and Exercise on SCI Tissue

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Introduction

Recovery after spinal cord injury (SCI) is inhibited by the scar tissue that develops at the site of the injury. The scar is formed by reactive astrocytes upregulating the expression of growth-inhibitory chondroitinase sulfate proteoglycans (CSPGs) including neurocan, aggrecan, and brevican[1].

A previous study within our lab determined that these changes are not limited to the injury site. Using western blots, we showed a significant increase in neurocan expression both at and far from the site of injury for four weeks post injury. We proposed that using enzymatic degradation on the scar matrix to regain plasticity may be an effective step towards recovery.

To examine this hypothesis, we performed contusion injuries on three groups of rats. Two of the groups were treated with chondroitinase (ChABC) either via intrathecal pump twice a week or via two µL microinjections into the grey matter one week after injury. Both groups were also treadmill trained every day to further promote recovery in SCI rats. It has been shown that treadmill training with weight support and manual assistance not only prevents muscle atrophy[2] but also increase the functional efficacy of the spared neurons[3].

The initial questions my project was meant to answer were how neurocan and glial fibrillary acidic protein (GFAP) were affected by the combinational therapy of chondroitinase and treadmill exercise. Neurocan has been shown to be elevated for four weeks post-injury[4] which is indicative of its influence in the injury response. GFAP is an intermediate filament that can be used as a cell marker for reactive CNS astrocytes[5]. By using Western blotting, I was able to observe how the amount of neurocan and GFAP varied at the epicenter of the lesion as well as at the lumbar region distal to the site.

Methods & Design

Spinal Cord Contusion Injury

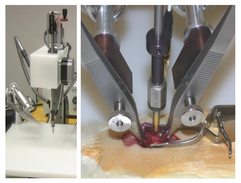
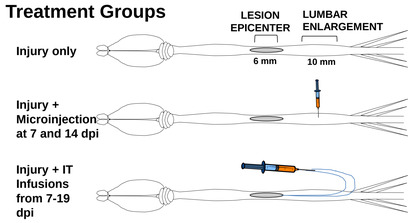


Figure 1. Severe spinal cord injury. Adult Female Sprague Dawley Rats (200-225 g) received a contusion injury using the Infinite Horizon (IH) Impactor at vertebral level T8. By 2 weeks post-injury, average BBB scores were 10-11. They could step, but had no forelimb/hindlimb coordination.

Treatment Groups



Treadmill Training

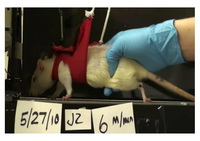
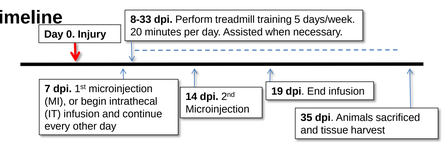


Figure 2. Injured rats were assigned to 3 groups for treatment (above) and the treadmill trained 5 days/week starting at 8 dpi with manual assistance for 20 minutes (Left). Sugar water was used as a incentive. Rats with improved motor coordination were set at faster paces.
At the end of the 4 weeks, behavioral recovery were analyzed by the BBB method, catwalk observation, and by activity boxes.

Study	Group/Treatment	Number of Subjects (for Western)
RR1	*Naïve*	N=4
	Injury Only	N=4
	Injury + treadmill	N=4
RR2	*Naïve*	N=4
	Injury Only	N=4
	Injury + MI	N=4
	Injury + IT	N=4

Timeline



Histology of Injury Epicenter and Lumbar Spinal Cord

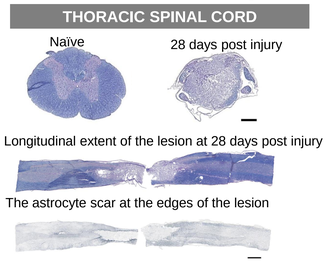


Figure 3. Representative tissue sections to illustrate the pathology of a spinal cord contusion injury. Top- cross sections of mid-thoracic cord before and after injury. EC/CV stain for myelin/Nissl. Scale bar = 200 µm. Bottom- longitudinal sections stained with EC/CV or antibodies to GFAP in astrocytes. Scale bar =1 mm.

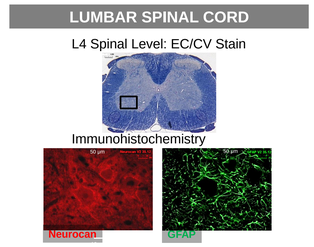
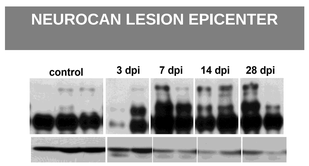


Figure 4. Tissue sections stained to illustrate the distribution of Neurocan and GFAP in the Lumbar spinal cord. Top- EC/CV section shows gray and white matter. Scale = 200 µm. Bottom- immunohistochemistry shows Neurocan in the extracellular matrix and perineuronal nets. GFAP stains astrocytes. Scale = 20 µm.

Effects of SCI on Neurocan Expression



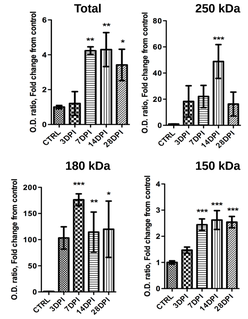


Figure 5. Above: WB of Neurocan at the epicenter; Right: Total neurocan expression is elevated 4-fold over control levels 7 dpi, and remains elevated. In the epicenter, there is an increase in normally present 150 kDa isoform, as well as increases in both full length 250 kDa isoform and a 180 kDa cleavage product.

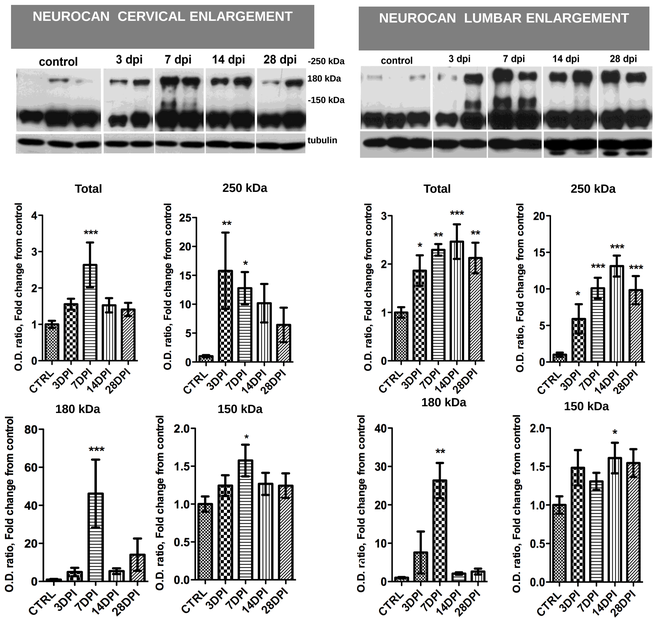


Figure 6. Western Blot Analysis of Neurocan at the Cervical and Lumbar Enlargements. In the cervical tissue, total neurocan expression is elevated 2.5-fold over control levels at 7 dpi, while the full length isoform is elevated 10-15 fold over controls. In the lumbar enlargement, total neurocan is increased 2-3 fold and expression of the full length isoform (250 kDa) is increased 7-14 fold over control values at all times. In both enlargements, this increase is not attributed to a corresponding increase in the normally present 150 kDa isoform.

Effects of Treadmill Training and ChABC on Neurocan at the Epicenter

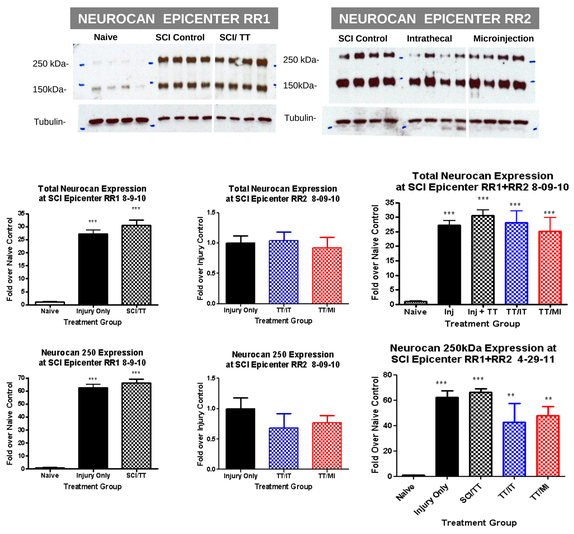


Figure 7. Western blot reveals a 25-30 fold increase of total neurocan levels at the lesion epicenter. No significant decrease of total neurocan levels in the treatment groups is observed. A 60-70 fold increase of full-length 250 kDa neurocan levels is observed for injury and all SCI/TT groups. A smaller increase of 40-60 fold is observed for the treatment groups. This suggests that treadmill training and ChABC treatment have little effect on the expression of neurocan **at the lesion site**.

Effects of Treadmill Training and ChABC on Neurocan in Lumbar Spinal Cord

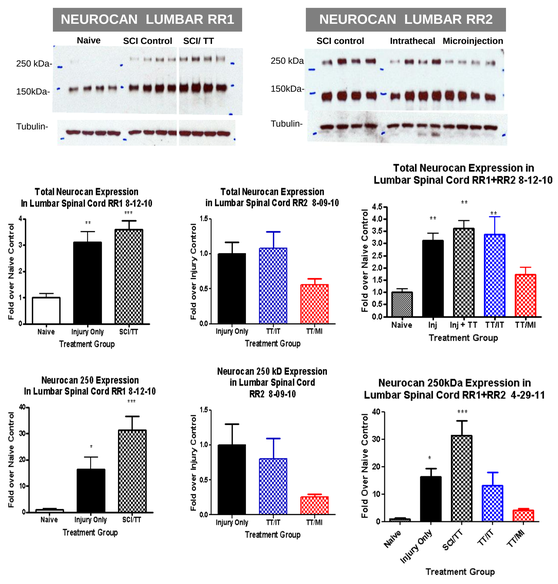


Figure 8. Western blot revealed a 3-4 fold increase in total neurocan levels in lumbar cord for injury, treadmill-trained, and intrathecal groups. Lumbar cord neurocan levels decreased to naïve levels for the microinjection group. Full length neurocan (250 kDa) was increased 20 fold after injury and further increased with treadmill training alone and reversed only in the microinjection group

Effects of Treadmill Training and ChABC on GFAP at Epicenter and Lumbar Cord

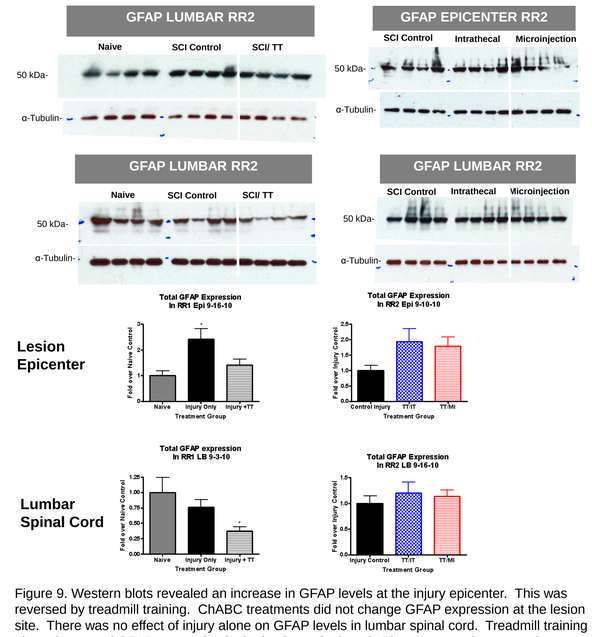


Figure 9. Western blots revealed an increase in GFAP levels at the injury epicenter. This was reversed by treadmill training. ChABC treatments did not change GFAP expression at the lesion site. There was no effect of injury alone on GFAP levels in lumbar spinal cord. Treadmill training alone decreased GFAP expression in the lumbar spinal cord. There was no decrease when treadmill training was combined with ChABC treatments.

Summary and Conclusions

- From this western blot study there are some important trends to notice. Most importantly, neurocan levels increased in the lumbar region after injury and decrease back to naïve levels after microinjection treatment. Full length neurocan (250kda) is also observed to decrease back to naïve levels in the lumbar region after microinjection. Therefore the microinjection method appears to have been the most effective treatment, at least in terms of enzymatic degradation of neurocan.

An increase of GFAP correlating with an increase of neurocan levels at the injury epicenter confirms the previous suspicion that reactive astrocytes are up-regulating neurocan to form the growth-inhibitory scar tissue along the lesion. Treadmill training alone seems to have lowered the response of reactive astrocytes at the epicenter. However, treadmill training plus treatment shows no difference in GFAP levels compared to injury. Perhaps digestion of the CSPGs in the extracellular matrix by chondroitinase causes the astrocytes to remain active.

Immunohistochemistry methods may reveal a significant difference in GFAP distribution around the borders of the injury, even if total expression in the spinal cord remains the same for treatment groups. Our lab will also be performing analysis to determine the effects of treatment on GFAP distribution and whether axon growth through the scar tissue or sprouting in lumbar spinal cord are altered after treatment. As the Western Blot data is combined with the behavior and IHC, we will gain a clear picture of how the treatment methods affect axon growth and functional recovery.

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Acknowledgements

PG received support for this work from the **Nishikawara Scholarship Fund for Physiology and Cell Biology at The Ohio State University**. The project was supported by NIH Grant NS043246 ISRT Grant STR100. The authors would like to thank Feng Qin Yin for technical expertise and assistance and Rebekah M. Richards for behavioral and histological data.