miRNA Antagomir Therapy to Promote Nerve Regeneration: An Experimental Study

Vadim Yerokhin BA; Patrick P. Han MD; Kennth Miller PhD
[Oklahoma State University, Center for Health Sciences, Tulsa, OK]
[St. John Neuroscience Institute, Tulsa, OK]

CNS 2016 ANNUAL MEETING San Diego CALIFORNIA SEPTEMBER 24-28, 2016

Learning Objectives

By the conclusion of this session, participants will be able to:

1) Describe changes in protein and miRNA expression in the spinal cord following peripheral nerve injury

2) Describe the approach to using miRNA inhibitors for accelerating peripheral nerve regeneration

3) Describe the feasibility of using these approaches in the clinical setting

Introduction

Peripheral nerve injury affects approximately 20 million Americans annually, costing the healthcare system over $150 billion each year. Although current therapies attempt to promote nerve regeneration, only 50% of patients fully regain motor and sensory function, while others retain poor motor control and neuropathic pain. Injured peripheral axons can regenerate, but this is rarely complete due to the slow rate of regeneration. A new therapeutic approach for accelerating peripheral nerve regeneration is needed. One approach is through micro RNA (miRNA) and anti-miRNA therapy, which has already proved fruitful in clinical application for other diseases. Nerve growth factor (NGF) is integral for nerve regeneration, while glutaminase (GLS) is involved in pain. Here, we clarify the role of miRNA let-7a and 23b in affecting NGF and GLS expression post-injury.

Methods

Male Sprague-Dawley rats were assigned into two groups: experimental (n=12), sham surgery (n=6). Sciatic nerve crush (SNC) was performed in the experimental and sham surgery groups, but not in the control group. Protein and miRNA expression data was collected at 7 days post-injury. Animals in the experimental group were subjected to sciatic nerve crush. The sciatic nerve was exposed and visualized, but not crushed in the sham group. We assessed the extent of nerve regeneration and changes in NGF and GLS expression in the dorsal root ganglia (DRG), the spinal cord (SC) and the sciatic nerve (SN) in response to SNC and compared these levels of expression to animals in the sham group. Changes in protein expression were measured using immunoblotting, while changes in miRNA expression were measured using real-time quantitative PCR.

Sciatic Nerve Crush Model

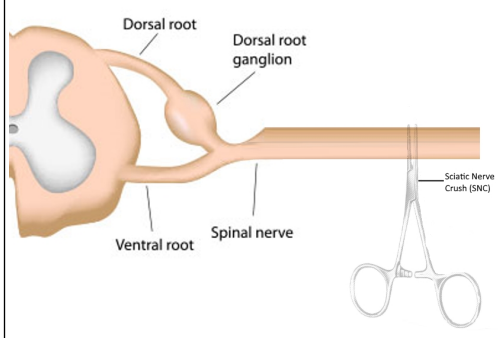

Figure 1. Sciatic nerve was crushed for 10 seconds using non-serrated hemostats

Results

miRNA let-7a and 23b levels correlate with expression of NGF and GLS protein, respectively, following sciatic nerve crush. A significant increase in let-7a and decrease in NGF at the spinal cord was observed at 1 and 7 days post-sciatic nerve crush. An increase in GLS protein and miR-23b miRNA were also observed, although these changes did not reach significance.

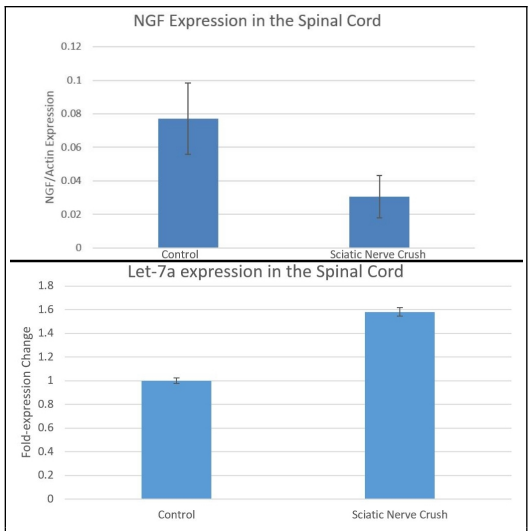

Figure 2. NGF protein and let7a miRNA expression in the spinal cord post-SNC

Protein and miRNA expression in the spinal cord following SNC

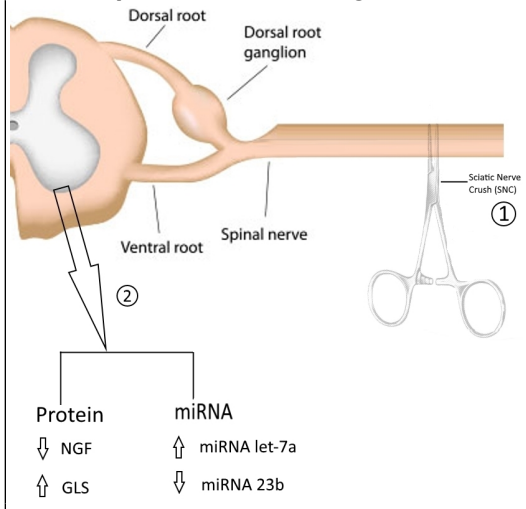

Figure 3. An •increase in let-7a and GLS and a decrease in NGF & miR-23b

Conclusions

Previous attempts to improve regeneration post-injury through delivery of NGF protein were found to carry detrimental side-effects. Given the pro-regenerative properties of the NGF and the role of GLS in pain, we are exploring a therapeutic approach to peripheral nerve injury that circumvents detrimental effects of protein delivery by altering protein expression at the translational level. Our findings on the relationship between peripheral nervous system (PNS) injury and expression of let-7a, miRNA-23b, NGF and GLS in the central nervous system (CNS) are important because they 1) reveal that NGF is expressed differently in the CNS vs. PNS post-injury and 2) open doors for a novel target to promote nerve regeneration through miRNA antagomir therapy.

References

Algonquin College. "A&P" 2012. Adapted from: http://lyceum.algonquincollege.com/lts/onlineCourses/anatomy/content/module7-2.htm.

Levi-Montalcini R, Hamburger V. Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool. 1951;116(2):321-61.

Miller KE, Akesson E, Seiger A. Nerve growth factor-induced stimulation of dorsal root ganglion/spinal cord co-grafts in oculo: enhanced survival and growth of CGRP-immunoreactive sensory neurons. Cell Tissue Res. 1999;298(2):243-53.

Miller KE, Hoffman EM, Sutharshan M, Schechter R. Glutamate pharmacology and metabolism in peripheral primary afferents: physiological and pathophysiological mechanisms. Pharmacol Ther. 2011;130(3):283-309.

Tian L, Prabhakaran MP, Ramakrishna S. Strategies for regeneration of components of nervous system: scaffolds, cells and biomolecules. Regen Biomater. 2015;2(1):31-45.

Walsh S, Midha R. Practical considerations concerning the use of stem cells for peripheral nerve repair. Neurosurg Focus. 2009;26(2):E2.

Woolf CJ, Safieh-Garabedian B, et al. (1994). Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience 62:327–331