Treating Spinal Cord Injury with Peripheral Nerve Grafts and Neurotrophin-3 Releasing Sutures

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Introduction

Peripheral nerve grafts (PNGs) in the spinal cord support axonal regeneration and functional recovery. We are testing a combination of PNGs and a sustained release of neurotrophin-3 (NT-3), that has been shown to induce axonal sprouting, promote axonal growth, and enhance neuroprotection. The sustained release of NT-3 is from biomineral coated sutures.Funding was obtained through a generous grant from the Bryon Riesch Paralysis Foundation.

Methods

NT-3 was bound to Biomineral coated 7-0 vicryl sutures. To test the release profile, the sutures were incubated in simulated body fluid at 37°C for 20 days. Every two days the amount of NT-3 in the release medium was quantified using an ELISA kit.

To test the biomineral coated sutures in vivo, five groups of Lewis rats received complete spinal cord injuries (SCI) at the T10 level: 1) controls; 2) PNGs; 3) PNGs plus biomineral coated sutures; 4) PNGs plus NT-3 injection; and 5) PNGs plus biomineral coated sutures releasing NT-3. Behavioral testing was done before grafting and weekly thereafter for eight weeks. To analyze axon regeneration axon tracers were injected into the motor cortex, brainstem (Biotinylated Dextran Amine: BDA) and sciatic nerves (Cholera Toxin B: CTB).

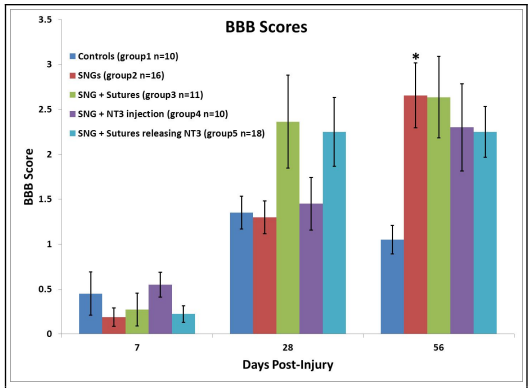

Figure 1: BBB scores. All treatment groups did better than controls. Only group 2 was significantly higher than group 1 on day 56. ($p<0.05$)

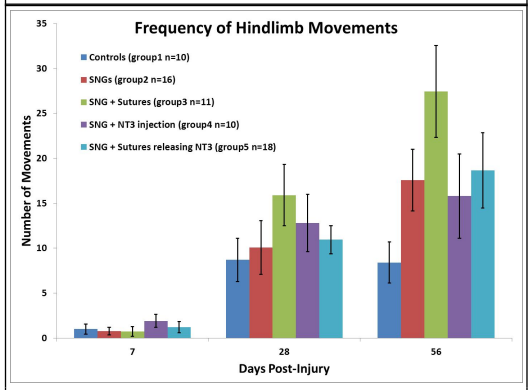

Figure 2. Frequency of hindlimb movements was improvement in all treated groups. This was not statistically significant

Results

In vitro there was a burst release of NT-3 followed by a sustained linear release for at least 20 days. Groups 2, 3 & 5 all had significantly higher ($P<0.05$) BBB scores than the controls 8 weeks after grafting. There was no significant difference in function between groups 2, 3, 4 and 5. Both BDA and CTB were observed in the grafts.

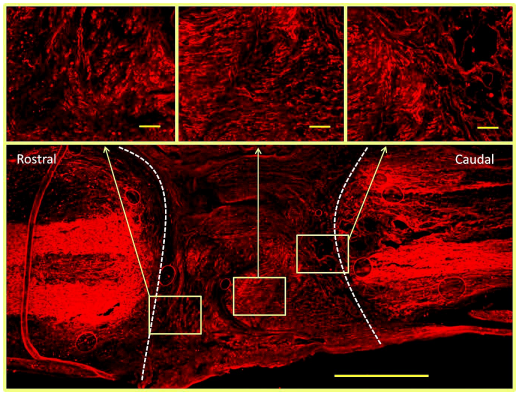

Figure 3. NF staining from a rat treated with SNGs and NT-3 injection reveals axons in the grafts. Dotted lines indicate interface. Top yellow scalebars: 100 um, bottom yellow scalebar: 1 mm.

Discussion The treated groups did better than the control group as far as the BBB scores. Sustained NT-3 release did not provide significant additional benefit to simple PNGs. We suspect that part of the lack of benefit was related to rejection. Inbred Lewis rats are supposed to be immunocompatible. We tested them for rejection and indeed some of them had positive CD8+ cells (Figure 5)and no neurofilaments. Future studies will involve testing NT-3 in other species (Sprague-Dawley rats) using immunosuppression, and in combination with other therapies like Chondroitinase ABC.

References 1. Cote, M.P., Hanna, A., Lemay, M.A., Ollivier-Lanvin, K., Santi, L., Miller, K., Monaghan, R. and Houle, J.D. (2010). Peripheral nerve grafts after cervical spinal cord injury in adult cats. Experimental neurology 225, 173-182. 2. Gumera, C., Rauck, B. and Wang, Y.D. (2011). Materials for central nervous system regeneration: bioactive cues. J Mater Chem 21, 7033-7051.

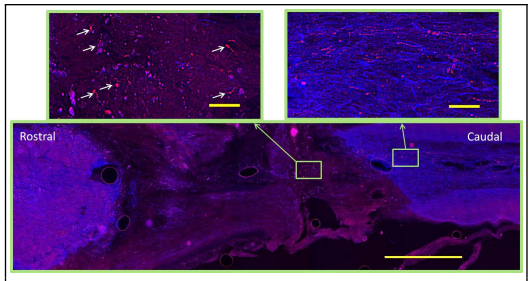

Figure 4. CTB labeled axons (red) extend over 1 mm into the graft in a rat treated with SNGs and NT-3 injection. Astrocytes (blue) outline the injury site. Top yellow scalebars: 100 um, bottom yellow scalebar: 1 mm.

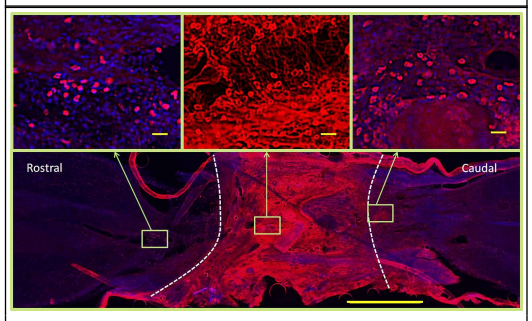

Figure 5. CD8+ cells are labeled on 30um thick horizontal sections from a rat that was treated with SNGs and no NT-3. There are several CD8+ cells migrating to the grafts and covering the entire grafted area, indicating graft rejection. CD8+ cells are labeled in red and nuclei are labeled with DAPI in blue. Dotted lines indicate graft spinal cord interface. Top yellow scalebars: 25 um, bottom yellow scalebar: 1 mm.

Conclusions

1. Peripheral nerve grafts support axonal growth after SCI. 2. NT3 use is safe in rats. 3. Combination therapy is probably key in treating SCI.