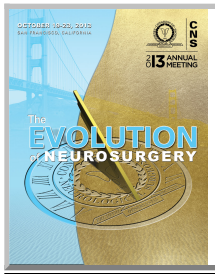

Spinal Grafting of Human Spinal Stem Cells in a Porcine L3 Contusion Model: Effect of High Dose Immunosuppression Treatment on Cell Graft Survival and Maturation in the Acutely Injured Spinal Cord.

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Learning Objectives

By the conclusion of this session, participants should be able to 1) Identify some of the potential barriers to cell replacement therapies (CRT) in patients with acute spinal cord injury (SCI), 2) Appreciate the means by which CRT may benefit SCI patients, 3) Describe some of the goals of future large animal and human trials necessary to bring CRT to fruition as an active therapeutic option.

Introduction

Spinal regenerative therapies, including cell replacement therapies (CRT), are rapidly gaining traction as viable treatments for acute and chronic spinal cord injury (SCI). Cell rejection, however has been demonstrated in xenograph, alloraft and even autograft models. Furthermore, the acutely injured spinal cord is a highly inflammatory, inhospitable environment for cell growth. Many authors have recommended waiting 7-10 days after injury in order to maximize cell survival, but others have demonstrated that longer wait times lead to greater glial scar formation and less therapeutic benefit to CRT. Before such a treatments can effectively be translated into clinical practice, large animal data is needed to characterize effective immunosuppression protocols and long-term survival of grafted cells in the potentially inhospitable milieu of the acutely injured spinal cord. In the present study, we characterize the survival and maturation of clinical grade human spinal stem cells (hNPCs) grafted in and around the injury epicenter using a porcine L3 contusion model.

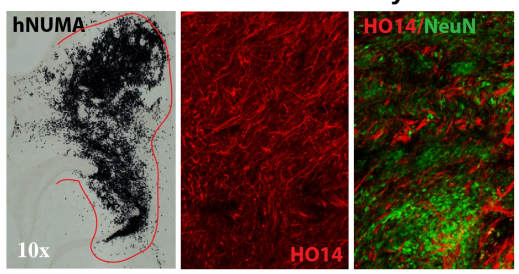
Methods

Isoflurane-anesthetized adult Gottingen-Minnesota minipigs (n=10) underwent 2-level laminectomies (L2-L5) followed by L3 spinal contusion using a 5-mm-diameter circular bar (peak force of 2.5kg at a velocity of 3cm/sec). At 24 hours post-injury, animals received 12 bilateral injections of hNPCs targeted in and around the injury epicenter. After cell grafting, animals were continuously immunosuppressed with tacrolimus (targeted blood level 50-60ng/ml) and mycophenolate mofetil (30mg/kg/day). During recovery, motor and sensory function were periodically monitored for 4 weeks. After survival, the presence of grafted cells was confirmed after staining spinal cord sections with a combination of human-specific (hNUMA, HO14, hNSE, hSYN) or non-specific (DCX, MAP2, CHAT, GFAP, APC) antibodies.

Results

In all cell-grafted animals, hNUMA-positive cells were readily identified. Numerous terminally differentiated grafted neurons with extensive axo-dendritic sprouting were seen; these exhibited hNSE and HO14 immunoreactivity. Similarly, a high density of hSYN-positive terminals derived from grafted neurons and residing in the vicinity of host neurons were also seen. A moderate degree of inflammatory change, as evidenced by the appearance of reactive astrocytes and microglia, was also identified.

Immunohistochemistry



Extensive human cell survival at 4 weeks after grafting at the T12 contusion site in immunosuppressed minipig. hNUMA-human specific nuclear antigen; HO14-human specific axonal neurofilament; NeuN-neron specific marker.

Conclusions

These data demonstrate that, using this immunosuppression protocol, xenograft cells grafted into the acutely injured spinal cord can survive a minimum of 4 weeks despite the inflammatory, post-traumatic environment. These results, as well as studies which have demonstrated long-distance axonal growth and synapse formation with improved functional outcome, are encouraging for the possibility of CRT becoming viable therapeutic options for patients with acute SCI.

This is, of course, a short-term survival study. Long-term studies are still necessary. Future studies will also seek to minimize immunosuppression in allograft and autograft models to support cell growth while minimizing immunosuppressive toxicity.

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