QS5

The Effect of Stem Cells and Local Tacrolimus on Neurite Extension

Sara Saffari, MD1,2, Tiam M. Saffari, MD1,2, Katelyn Chan, MASc BioSc3, Gregory H. Borschel, MD3, Alexander Y. Shin, MD1.

1Mayo Clinic, Rochester, MN, USA, 2Radboud, Nijmegen, Netherlands, 3The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

Purpose: Application of mesenchymal stem cells (MSCs) or tacrolimus (FK506), an FDA approved immunosuppressant, to nerve grafts has been a topic of interest to enhance peripheral nerve regeneration. The aim of this study was to investigate the combined effect of MSCs and local delivery of FK506 on nerve regeneration when applied to nerve autografts and decellularized nerve allografts.

Methods: A three-dimensional (3D) in vitro compartmented cell culture system, validated by Tajdaran et al (2019), consisted of rat neonatal dorsal root ganglion (DRG) adjacent to rat nerve autograft or decellularized allograft. This model was used to evaluate regenerating neurites from the DRG into the peripheral nerve scaffold. Nerve autografts and decellularized allografts were augmented with (i) dynamic undifferentiated MSC seeding, (ii) local application of FK506 (100 ng/mL) or (iii) both (N=9/group). Local application was ensured by isolating the central system (i.e. DRG side) from the peripheral system (i.e. nerve graft side), where treatment was applied. After 48-hours of incubation, DRG-nerve graft constructs were collected, fixed, sectioned and stained against neurofilament-160 to measure neurite extension. CD90 staining was used to confirm stem cell characterization.

Results: All grafts treated with MSCs confirmed CD90 expression. Compared to untreated autografts, neurite extension in autografts treated with FK506 and autografts treated with MSCs and FK506 combined were found superior (P<0.001 and P=0.0001, respectively), and comparable to autografts treated with MSCs (P=0.12). Compared to untreated allografts, allografts treated with FK506, and allografts treated with MSCs and FK506 were found superior (P<0.001 and P=0.0001, respectively), and allografts treated with MSCs were found comparable (P=0.09). All autograft groups were found superior compared to their respective allograft treatment group (P<0.05). Solely allografts receiving combined treatment were found superior to untreated autografts (P<0.05).

Conclusion: MSCs or FK506 treatment improved neurite outgrowth and when combined, this resulted in significant synergistic neurite extension in both autografts and allografts in comparable patterns. Schematic overview of 3D compartmented cell culture system for isolated evaluation of treatment with MSCs and local FK506 in vitro. A 3.5 mm autograft or allograft with or without undifferentiated MSC seeding is attached to a DRG. DRG-nerve graft constructs are placed through a silicone isolator in the middle of a 24-wells plate to isolate the DRG from the nerve graft. FK506 containing media was added to the nerve graft side.

QS7

Physiologic Signaling of the Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI) During Volitional Behavior

Shelby R. Svientek, M.D.1, Jarred Bratley, B.S.2, Amir Dehdashtian, MD, MPH1, Carrie Kubiak, MD1, Paul Cederna, MD1, Stephen Kemp, PhD1.

1University of Michigan, Ann Arbor, MI, USA, 2Wayne State University, Detroit, MI, USA.

Purpose: Exoskeletons have become a promising device to restore extremity function to those with limb weakness. However, these devices have not become widely adopted due to the inadequacy of current nerve interfacing methods. The Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI) was developed as a potential solution to this problem. Consisting of a segment of autologous free muscle secured around an intact nerve, the MC-RPNI is able to regenerate and reinnervate, amplifying its contained nerve’s signals through generation of EMG signals. These amplified, high-fidelity signals can then be used to intuitively and accurately control exoskeletons. The purpose of this study was to characterize MC-RPNI physiologic signaling during volitional behavior and determine long-term effects on the associated nerve.

Methods: Eighteen rats were randomly assigned to one of three groups: (1) sham surgery/control; (2) nerve
TRANSECTION; and (3) MC-RPNI. MC-RPNIs were surgically fabricated by wrapping isogenic donor muscle graft circumferentially around the common peroneal (CP) nerve. At six months, CP nerve cuff electrodes were implanted in Groups 1 and 2, and patch electrodes were placed on all MC-RPNIs. Gait analysis and electrophysiological evaluations were performed the following day. Rats were trained to walk on a treadmill, and electrode recordings were obtained and correlated with gait videocapture. All rats had their proximal CP nerve stimulated, with efferent signals obtained at (1) downstream nerve (CSNAP), (2) MC-RPNI (CMAP), and (3) downstream-innervated extensor digitorum longus (EDL) muscle (CMAP). EDL muscle force testing was also performed following stimulation of the CP nerve.

Results: All MC-RPNIs remained viable and demonstrated appropriate regeneration, revascularization, and reinnervation on histology. The MC-RPNI was found to generate large-amplitude CMAPs (2.77±0.926 mV), amplifying its associated nerve’s signal (50.5±8.18 µV) over 50-fold on average. The MC-RPNI was not found to affect muscle function when evaluating downstream-innervated EDL CMAPs (control: 13.4±2.33 mV vs MC-RPNI: 14.1±1.44 mV) or maximal twitch force (control: 562±70.8 mN vs MC-RPNI: 653±34.6 mN). On gait analysis, recordings from the MC-RPNI correlated with the toe-off phase of gait; for the control groups, nerve signaling could not be differentiated from background noise. When comparing MC-RPNI to control animal gait, no significant differences were noted on qualitative or joint-angle analysis.

Conclusion: The MC-RPNI has the ability to chronically amplify physiologic nerve signals from intact peripheral nerves by several magnitudes while avoiding functional impairment of downstream-innervated muscle. This amplification has the capability to facilitate high accuracy detection of motor intent in order to intuitively and reliably control advanced exoskeleton devices.

QS8

The Roles of the TrkA and p75NTR NGF Receptors in Corneal Wound Healing

Kiana Tajdaran, Konstantin Feinberg, Seyed Kaveh Mirmoeini, Jennifer Zhang, Tessa Gordon, Gregory Borschel

University of Toronto, Toronto, ON, Canada, The Hospital for Sick Children, Toronto, ON, Canada, Indiana University, Indianapolis, Indiana

Purpose: The cornea is the window through which we see the world and is one of the most densely innervated structures in the body. Besides providing protective sensory input, corneal nerves have been postulated to stimulate limbal stem cells (LSCs), hence governing corneal epithelial maintenance and recovery. Loss of corneal innervation, through injury, diabetes, tumors, infections, and even improper contact lens use, leads to neurotrophic keratopathy (NK), a degenerative corneal disease that is characterized by corneal epithelial breakdown, scarring, and permanent vision loss. The only non-invasive treatment option for NK is the human recombinant nerve growth factor (rhNGF), but the short half-life of exogenous neurotrophins-based therapies make this therapeutic approach less effective. Development of the small molecule ligands for neurotrophins receptors that have better pharmacokinetics and plasma stability showed promising results in the treatment of several neurodegenerative conditions in the recent years. In this study, we were prompted to investigate the molecular mechanism of NK and the role of NGF receptors, TrkA and p75NTR, in corneal healing. We hypothesized that TrkA inhibition would delay corneal wound healing and p75NTR inhibition accelerates corneal healing. This knowledge will lay the basis for a new non-invasive approach for NK.

Methods: For this experiment, we took advantage of commercially available Ntrk1 mutant mice, which allow for pharmacological inhibition of TrkA receptor with an inhibitor known as not mammalian kinase inhibitor (1-NM-PP1). Ntrk1 mice (n=20) were divided into three groups, which received saline injection as a control. In one experimental group animals were received TrkA inhibitor and the other group received both TrkA and p75 inhibitor for 5 days. On day six we removed the corneal epithelium with a 0.5 mm rotating brush. To measure epithelial healing, we performed digital imaging of fluorescein staining daily for four days after injury. We then harvested the corneas for immunofluorescent and biochemical analyses.

Results: Our results show a significant delay in corneal epithelial healing following TrkA inhibition and acceleration in corneal healing after p75NTR inhibition.

Conclusion: A selective TrkA agonist or p75NTR inhibitors could be a new therapeutic approach for NK.