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Self-assembling peptides optimize the post-traumatic milieu and synergistically enhance the effects of neural stem cell therapy after cervical spinal cord injury

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ABSTRACT

Introduction: The hostile environment after spinal cord injury (SCI) can compromise effects of regenerative therapies. We hypothesized that optimizing the post-traumatic environment with QL6 self-assembling peptides (SAPs) before neural precursor cell (NPC) transplantation would improve cell survival, differentiation and functional recovery.
Methods: A total of 90 Wistar rats received a clip-compression SCI at C7. Within each of two study arms, animals were randomized into 5 groups (NPC, SAP, NPC + SAP, vehicle, and sham). SAPs and NPCs were injected into the spinal cord 1 day and 14 days post-injury, respectively. Animals received growth factors over 7 days and were immunosuppressed. Rats were sacrificed at 4 weeks and sections of the cervical spinal cord prepared for immunohistochemistry (first study arm). Neurological function was assessed weekly for 8 weeks using a battery of behavioral tests. Nine weeks post-SCI, the corticospinal tract was assessed using fiber-tracking (second arm).
Results: SAP-treated animals had significantly more surviving NPCs which showed increased differentiation to neurons and oligodendrocytes compared to controls. SAPs alone or in combination with NPCs resulted in smaller intramedullary cysts and larger volume of preserved tissue compared to other groups. The combined treatment group showed reduced astrogliosis and chondroitin sulfate proteoglycan deposition. Synaptic connectivity was increased in the NPC and combined treatment groups. Corticospinal tract preservation and behavioral outcomes improved with combinatorial treatment.
Conclusion: Injecting SAPs after SCI enhances subsequent NPC survival, integration and differentiation and improves functional recovery.

Statement of Significance

The hostile environment after spinal cord injury (SCI) can compromise effects of regenerative therapies. We hypothesized that improving this environment with self-assembling peptides (SAPs) before neural precursor cell (NPC) transplantation would support their beneficial effects. SAPs assemble once injected, providing a supportive scaffold for repair and regeneration. We investigated this in a rat model of spinal cord injury.
More NPCs survived in SAP-treated animals and these showed increased differentiation compared to controls. SAPS alone or in combination with NPCs resulted in smaller cysts and larger volume of preserved tissue with the combined treatment also reducing scarring and improving behavioral outcomes.
Overall, injection of SAPs was shown to improve the efficacy of NPC treatment, a promising finding for those with SCIs.

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1. Introduction

Cervical spinal cord injury (SCI) is a disastrous event, which can lead to lifelong disability and loss of independence. Cervical lesions can cause paresis or paralysis of the upper and lower extremities and can additionally affect bladder and bowel function, respiration, and trunk stability. More than 50% of spine injuries involve the cervical region [1–3]; which is distinct from the thoracolumbar region in both anatomy and pathophysiology. In particular, differences exist in vascular organization, volume of gray and white matter, and neural segmentation. This makes cervical trauma models highly clinically-relevant, particularly when moving towards translation.

Following severe SCI, a secondary injury cascade is initiated which results in loss of neurons, release of cytotoxic factors, demyelination, and development of intramedullary cystic cavitation. Although surviving axons persist in a subpial rim of white matter, possessing the potential to support regeneration, the hostile microenvironment limits endogenous repair [7]. Furthermore, cystic cavities and deposited astroglial/proteoglycan scar form a physical barrier to axonal outgrowth and cell migration [4–6,8–10].

Exogenous cell transplantation thus emerges as a promising treatment strategy to overcome structural tissue-damage and generate functional recovery. Stem cells and their progeny may promote neuroprotection by modifying the toxic microenvironment, inhibiting inflammatory signaling, and releasing growth factors [11]. Furthermore, in the sub-acute stage transplanted cells can support host axon regrowth by reducing neurite growth-inhibitory substances, releasing trophic factors, enhancing remyelination of axons and supplying an extracellular matrix [12]. Of particular interest are neural precursor cells (NPCs) which can differentiate into both neurons and glia [13]. NPCs have been shown to promote remyelination [14,15] and support functional recovery [16], making them very promising for the treatment of spinal cord injuries. Unfortunately, transplanted cells have poor survival rates. Efforts are currently underway to improve engraftment and differentiation through combinatorial approaches with growth factors, sonic hedgehog proteins and chondroitinase [17,18].

Scaffolds, such as collagens, fibrin or plasma, represent an exciting bioengineered strategy to bridge the lesion cavity and shape the inhibitory post-traumatic microenvironment [19]. Agarose hydrogels or alginates reduce astroglial and fibrotic scarring and serve as a matrix to deliver drugs or growth factors, both supporting outgrowth and regeneration of axons [20–23]. NeuroGel (poly-(N-[2-hydroxypropyl]methacrylamid)-hydrogel) additionally supports angiogenesis, whereas fibronectin shows convincing effects of orientated growth of axons within the damaged spinal cord [24–27]. Some studies also used multicomponent polymers or collagens in order to both provide a scaffold and to transport or deliver factors (e.g., neurotrophin-3) or cells (e.g., olfactory nerve ensheathing cells or neural stem cells) within the damaged spinal cord [28–30].

In recent years, self-assembling peptides (SAPs) have been developed for tissue engineering and factor delivery. As a novel treatment approach, SAPs can be injected directly into the epicenter of the lesion to self-assemble into 3D nanofibers which scaffold the intramedullary cavity, modify the microenvironment, and serve as a structural framework for the integration and axonal outgrowth of transplanted cells [31,32]. In SCI treatment, SAPs containing IKVAV (IKVAV PA) have been shown to reduce astrogliosis and cell death in addition to promoting regeneration [33]. Another SAP, RADA 16-1, has been shown to bridge the injured spinal cord and elicit axon regeneration [34]. Since RADA 16-1 is acidic, with a pH of 3–4, it cannot be applied directly to nervous tissue as it causes inflammation and cysts making pre-buffering mandatory [34]. $K_2(QL)_6K_2$ (QL6) is a novel SAP introduced by Dong et al. and provided by Covidien (Medtronic Inc.) [35,36]. It is a multidomain peptide (MDP) with an ABA structure. The central B block consists of a variable number of glutamine and leucine (QL). The alternating hydrophilic and hydrophobic pattern allow all glutamine side chains to lie on one side creating a driving force for the hydrophobic faces to pack against one another forming a hydrophobic sandwich. To mitigate the hydrophobic character and thus, poor solubility, the A block containing a variable number of charged amino acids (lysine) was added to both termini [35]. The $K_2(QL)_6K_2$ type is soluble in water at a pH of 7.4 and showed a very strong β-sheet conformation and a population of nanofibers with uniform diameter (6 ± 1 nm), controlled length (120 ± 30 nm), and no amorphous aggregates [35]. On the other hand, adding a fourth lysine on the terminal endings totally changes the secondary structure from a β-sheet to an unstable α-helix conformation [35]. The $K_2(QL)_6K_2$ (QL6) β-sheet conformation is stable and may find use in understanding and treatment of various diseases caused by protein aggregation or as nanostructured scaffold for bioengineering [35]. When applied alone, QL6 reduces post-traumatic apoptosis, inflammation and astrogliosis leading to electrophysiological and behavioral improvements after spinal cord injury [36]. Furthermore, QL6 has been shown to support co-transplanted NPCs and to biodegrade over several weeks making it an ideal candidate in SCI [36,41].

With this background, we hypothesized that QL6 supports NPC survival and differentiation by modifying the microenvironment and that this effect can be further enhanced by creating a lead time between QL6 injection and NPC transplantation. In the current study, we examined whether pre-treatment with QL6 SAPs injected into the injured spinal cord tissue immediately after SCI positively shapes the hostile microenvironment to support subsequent NPC therapy. By using this novel strategy, we expect enhanced NPC survival, integration and differentiation with QL6 pretreatment. Furthermore, we anticipate reduced inflammation and tissue scarring and attenuation of neurological deficits.

2. Materials & methods

2.1. Animals

A total of 90 female Wistar rats (250 g; Charles River Laboratories, Wilmington, MA) were used. All experimental protocols were approved by the animal care committee of the University Health Network in accordance with the policies established in the *Guide to the Care and Use of Experimental Animals prepared by the Canadian Council of Animal Care*.

2.2. Experimental groups

This study contained two major study-arms with different survival times following SCI (28 days and 9 weeks). Each study arm was composed of 5 groups:

Group I: vehicle + vehicle.
Group II: SAPs + vehicle.
Group III: vehicle + NPCs.
Group IV: SAPs + NPCs.
Group V: sham operated animals (laminectomy only without SCI).

Following SCI, animals were randomized into their individual treatment groups by drawing lots. SAPs or vehicle were injected into the intramedullary cavity 1 day after SCI. 14 days after SCI, NPCs or vehicle were transplanted into the perilesional area. All