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A self-assembling peptide reduces glial scarring, attenuates post-traumatic inflammation and promotes neurological recovery following spinal cord injury ☆

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ABSTRACT

The pathophysiology of spinal cord injury (SCI) involves post-traumatic inflammation and glial scarring which interfere with repair and recovery. Self-assembling peptides (SAPs) are molecules designed for tissue engineering. Here, we tested the performance of $K_2(QL)_6K_2$ (QL6), a SAP that attenuates inflammation and glial scarring, and facilitates functional recovery. We injected QL6 into the spinal cord tissue of rats 24 h after clip compression SCI. QL6 led to a significant reduction in post-traumatic apoptosis, inflammation and astrogliosis. It also resulted in significant tissue preservation as determined by quantitative histomorphometry. Furthermore, QL6 promoted axonal preservation/regeneration, demonstrated by BDA anterograde and Fluorogold retrograde tracing. In vitro experiments found that a QL6 scaffold enhanced neuronal differentiation and suppressed astrocytic development. The electrophysiology confirmed that QL6 led to significant functional improvement of axons, including increased conduction velocity, reduced refractoriness and enhanced high-frequency conduction. These neuroanatomical and electrophysiological improvements were associated with significant neurobehavioral recovery as assessed by the Basso–Beattie–Bresnahan technique. As the first detailed examination of the pathophysiological properties of QL6 in SCI, this work reveals the therapeutic potential of SAPs, and may suggest an approach for the reconstruction of the injured spinal cord.

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1. Introduction

Neurotrauma, including spinal cord injury (SCI), is a leading cause of neurological disability and death. The pathophysiology of SCI involves a primary mechanical injury followed by a series of secondary molecular and cellular changes [1]. The inherent challenge of developing therapies aimed at treating SCI is rooted in the complexity of its pathobiology, which includes invasion of inflammatory agents, ongoing apoptosis of neural cells, demyelination, formation of cavities or cysts, glial scarring and the loss of complex neural circuitry [2]. The combined presence of cavitation and glial scarring acts as a physical and chemical barrier to repair, plasticity and regeneration. Current therapeutic strategies for acute SCI are chiefly aimed at preventing tissue loss with pharmacological agents. No interventions have yet been developed to reconstruct post-traumatic cyst cavities. Hence, it is attractive to develop a tissue-engineered scaffold which could act as a bridge for endogenous cell migration, axonal elongation and plasticity.

In the past decade, self-assembling peptides (SAPs) have been developed for tissue engineering and protein delivery [3]. Synthetic SAPs can self-assemble into nanofibers in situ under physiological conditions, without apparent immune response [4]. These properties make SAPs appealing for application in SCI because they can be injected directly into the lesion site, thus minimizing the damage to the cord which can occur with the implantation of solid scaffolds. Liquid SAPs can readily fill the cavities, regardless of their size and shape, and become integrated with host tissue after self-assembling to a hydrogel. The use of SAPs is a novel approach in providing a nanofibrillar biocompatible structure, similar to the native extracellular matrix. This direct contact between nanofibers and the extracellular matrix may be critical in facilitating

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cell–scaffold interaction [5,6]. SAP nanofiber scaffolds have become increasingly important, not only in studying the three-dimensional microenvironment, but also in developing approaches for a wide range of innovative medical technologies, including regenerative medicine [5]. It has been reported that after SCI, in vivo treatment with IKVAV containing SAP amphiphile (IKVAV PA) reduces astrogliosis and cell death and promotes regeneration of both descending motor fibers and ascending sensory fibers [7]. In this study, it was necessary to promote PA bioactivity by incorporating the neurite-promoting laminin epitope IKVAV into the molecular design of the scaffold. Another SAP substance found in the literature is RADA16-I (Ac-RADARADARADARADA-$CONH_2$), this peptide was found to bridge the injured spinal cord, elicit axon regeneration [8] and reduce the glial reaction and inflammation in surrounding brain tissue [9]. In a recent paper, a bone marrow homing peptide, RADA16-I SAP, functionalized by combining with a bioactive peptide, PFSSTKT (BMHP1), was found to induce a favorable matrix remodeling process and provide physical and trophic support to spinal cord tissue regrowth, cellular infiltration and axon regeneration/sprouting [10]. However, RADA16-I has a pH of 3–4; when applied directly onto nervous tissue, this degree of acidity may cause tissue damage. Guo et al. reported that untreated RADA16-I with low pH values could be detrimental in that they cause inflammation in the host spinal cord tissue, creating distinct gaps and cysts surrounding the implants [8]. Therefore, there are certain limitations associated with the use of RADA16-I, notably its intrinsically low pH and the resultant need for prebuffering to counteract the acidity of the SAP.

$K_2(QL)_6K_2$ (QL6) is a novel SAP introduced by Dong et al. [11] and provided by Covidien. It is important to note that QL6 self-assembles into β-sheets at neutral pH, a feature that is rare among peptides, which generally form insoluble β-sheet assemblies [11]. The present study was designed to determine the effects of QL6 in the reconstruction of the injured spinal cord when administered at the clinically relevant time point of 24 h post-SCI. We reported that, without any bioactive adjunct, QL6 can provide favorable support for neuronal growth and functional repair post-SCI, as shown by combined neuroanatomical, electrophysiological and neurobehavioral data. These observations suggest that the SAP QL6 is a promising biomaterial for central nervous system injury.

2. Materials and methods

2.1. Animal SCI and intraspinal microinjection

All animal experiments were conducted with approval from the Animal Care Committee, University Health Network (Toronto, Canada). The aneurysm clip compression/contusion model of SCI used in our laboratory has been characterized extensively and described previously [12,13]. Briefly, adult female Wistar rats (250–300 g; Charles River, Montreal, Canada) received laminectomies of mid-thoracic vertebral segments T6–T7. A modified clip calibrated to a closing force of 35 g was applied extradurally to the cord for a duration of 1 min. The animals were divided into two groups in a randomized and blinded manner, namely the QL6 treatment group and a saline control group. We chose a QL6 concentration of 1% (w/v) for gel formation that can mimic extracellular matrix [7,11]. Gelain et al. [14] reported that a SAP concentration of 1% (w/v) yielded a hydrogel scaffold stiffness similar to that of the spinal cord. The opacity and mechanical stiffness of the hydrogels increase with increasing peptide concentration. 1% (w/v) peptide concentration demonstrated a medium fiber density under scanning electron microscopy observation, which could have a stable nanostructure (unpublished data).

Using a stereotaxic frame and glass capillary needle connected to a Hamilton microsyringe, a total of 10 μl was injected into the dorsal spinal cord 24 h after SCI. Two intraspinal injections were made bilaterally rostral and caudal to the injury site starting at 2 mm below the dorsal surface and then at intervals of 0.5 mm (three sites), with 1.67 μl being injected at each interval amounting to a total dose of 5 μl at each stump (Fig. 1B). The injection rate was 0.5 μl per minute and, at the end of injection, the capillary was left in the cord for at least 1 min to allow diffusion from the injection site.

2.2. Electron microscopic characterization of the scaffolds

2.2.1. Transmission electron microscopy (TEM)

QL6 samples were applied to carbon-coated copper grids, and were allowed to adsorb for 1 min. The grids were then stained by 2% phosphotungstic acid for 30 s for negative staining. The stained grids were allowed to air dry before imaging. Electron microscopy images were obtained using a Hitachi H-7000 transmission electron microscope at an 75 kV accelerating voltage.

2.2.2. Scanning electron microscopy (SEM)

Samples were soaked in 5% glutaraldehyde at 4 °C for 2 h, slowly dehydrated in 10% increment steps of ethanol for 5 min each, and placed in a pressurized liquid CO_2 siphon for 1 h using a CO_2 critical point dryer. Scaffolds were coated with gold with a sputter coater, and images were taken with a Hitachi S-3400N scanning electron microscope.

2.3. Histological processing and tissue sparing assessment

Animals were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The tissues were then cryoprotected in 25% sucrose in PBS. A 2 cm length of the spinal cord centered at the injury site was embedded in tissue-embedding medium. The injured segment was snap frozen and sectioned on a cryostat (Leica CM3050S). Serial spinal cord sections at 500 μm intervals were stained with myelin-selective pigment luxol fast blue (LFB) and the cellular stain hematoxylin–eosin (HE) to identify the injury epicenter.

2.3.1. Assessment of tissue sparing at the injury site

Tissue sparing was analyzed 8 weeks after SCI, at the center of the lesion, 2 mm rostral and 2 mm caudal to the epicenter. Sections were stained with LFB-HE. The measurements were carried out on coded slides using ImageJ software (NIH). Cross-sectional residual tissue was normalized with respect to total cross-sectional area and the areas were calculated every 500 μm within the rostrocaudal boundaries of the injury site.

2.4. GFAP and Iba-1immunohistochemical procedures and image analysis

The following primary antibodies were used: mouse anti-glial fibrillary acidic protein (GFAP) (1:500; Chemicon International, Inc., Temecula, CA) for astrocytes, mouse anti-Iba-1 (1:500, Wako, Japan) for macrophages/microglia [15]. The sections were rinsed three times in PBS after primary antibody incubation and incubated with fluorescent Alexa 568 goat anti-mouse/rabbit secondary antibody (1:400; Invitrogen, Burlington, ON) for 1 h. The sections were rinsed three times with PBS and coverslipped with Mowiol mounting medium containing DAPI (Vector Laboratories, Inc., Burlingame, ON) to counterstain the nuclei. The images were taken using a Leica epifluorescence microscope.

For immunodensity measurements of GFAP and Iba-1, four sections were selected at the epicenter, as well as at 1 and 2 mm (two rostral and two caudal) from the injury epicenter in each animal. We photographed the entire transverse section at 10× primary

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