ISSN: 2321-8819 (Online) 2348-7186 (Print) Impact Factor: 1.498 Vol. 5, Issue 10, October 2017

The Role of Self-Accumulated Peptide Amphiphile in Spinal Cord Injury Functional Reclamation

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Abstract: Injection into an experimentally injured spinal cord of a self-assembling peptide amphiphile (PA) that displays an IKVAV epitope reduced glial scarring and improved functional reclamation (Tysseling-Mattiace et al., 2008). Injection of a material that lacked this epitope did not alter outcome suggesting that signaling by the IKVAV epitope was central to the beneficial effects of IKVAV-PA. However the mechanical properties of implanted materials may also alter tissue and cell behavior in vivo (Discher et al., 2005). We therefore explored whether the mechanical properties of PAs might affect outcome after spinal cord injury. By treating animals with a spinal cord injury with different PAs that varied in their mechanical properties without epitope presentation, we found that the beneficial effects of the PAs are primarily dependent upon the presentation of a bioactive epitope presentation rather than the mechanical properties of the PA scaffold.

Keywords: Self-assembling peptide amphiphile, functional recovery, spinal cord injury

INTRODUCTION

Spinal cord injury is a disease with no effective therapeutic intervention at the present time. Previously, our lab had reported that we were able to improve SCI functional reclamation by injecting self-assembling peptide amphiphile (PA) that displays an IKVAV epitope into the injured spinal cord (Tysseling-Mattiace et al., 2008). Injection of IKVAV-PA reduced glial scar formation, promoted corticospinal tract regeneration in the spinal cord, and enhanced behavioral outcome. The basic properties of peptide amphiphiles are described earlier in this paper. IKVAV (isoleucine-lysine-valine-alanine-valine) is a natural sequence found in the laminin A chain which is thought to mediate the bioactivity of laminin (Tashiro et al., 1989, Agius et al., 1996).

However there are studies suggesting that the mechanical properties of matrix material also contributing to their biological effects. As reviewed by Discher et al (2005), extracellular matrix stiffness is sensed through cell membrane anchorage proteins such as integrin, affecting cell proliferation, differentiation and migration. Specifically in the nervous system, studies on neurite growth have shown that the stiffness of hydrogels mediates their effects on the rate of neurite outgrowth (Man et al., 2011, Chen et al., 2013).

Ever since we found the beneficial effects of IKVAV PA for the treatment of spinal cord injury (Tysseling-Mattiace et al., 2008), we have been working with our collaborators to improve and optimize the PA based SCI treatment. An important issue within this effort was to understand the determinant factors of the effects of IKVAV PA.

MATERIAL AND METHOD

RAt spinal rope damage and PA infusion:

Every creature method was performed as per the Public Health Service Policy on Humane Care and Use of Laboratory Animals and all techniques were endorsed by the Medical University Institutional Animal Care and Use Committee. Female Longs Evans Rats (two months of age) were anesthetized by the inward breath of 2.5% isoflurane soporific in 100% oxygen managed by a VetEquip Rodent anesthesia machine. A T11 vertebral laminectomy was performed to uncover the spinal string. The spinal string was harmed utilizing an IH-0400 Spinal Cord impactor (Precision Systems) with a 1.25mm tip with a 2.50mm tip with 185 Kdynes of power and 60s of stay time. Skin was sutured utilizing AUTOCLIPs (9 mm; BD Biosciences). For postoperative care, creatures were continued a warming cushion for 24 h to keep up body temperature. After surgery, rats were given Buprenex (2.5mg/kg, s.c.) and Baytril (5 mg/kg, s.c.) to limit uneasiness and disease. Bladders were physically communicated twice day by day. A 5 day Baytril treatment course (5 mg/kg day by day, s.c.) was begun in case of hematuria.

Dad (1% watery arrangement) or vehicle was infused 48 h after SCI utilizing borosilicate glass narrow micropipettes (Sutter Instruments, Novato, CA) (external width, 100 μm). The vessels were stacked onto a Hamilton syringe utilizing a female luer connector (World Precision Instruments, Sarasota, FL) controlled by a Micro4 microsyringe pump controller (World Precision Instruments).

The amphiphile was weakened 1:1 with a 580 µM arrangement of glucose just before infusion and stacked into the slim. Under Avertin anesthesia, the autoclips were expelled and the damage site was uncovered. The micropipette was embedded to a profundity of 750µm measured from the dorsal surface of the string for mice and a profundity of 1250 µm, and 5.0 µl of the weakened amphiphile arrangement or vehicle was infused at 1 µl/min. The micropipette was pulled back at interims of 250 µm to leave a trail (ventral to dorsal) of the PA in the string. Toward the finish of the infusion, the slim tip was left in the line for an extra 1 min, after which the pipette was pulled back and the injury shut. For all analyses, the experimenters were kept blinded to the personality of the creatures.

Statistical analyses:

For comparison of behavioral BBB scoring between different injection groups, two-way ANOVA test was performed.

RESULTS AND CONCLUSION

To investigate this possibility that the stiffness of PA supplied to the injury site influences outcome after spinal cord injury, we tested several PAs without bioactive epitopes that differed in their mechanical properties and compare them to a glucose vehicle. One of these PA ("linear PA") has the same molecular backbone as IKVAV PA and should provide the same stiffness support as the IKVAV PA. The second PA has a similar molecular composition but a branched molecular structure design that increased the stiffness of the scaffold formed after gelation.

We used our standardized impaction platform to injure adult rats. Two days after the injury, the severely injured rats (recognized as BBB score of 0 on both hind limbs (Basso et al., 1996, Joshi and Fehlings, 2002b)) were injected with either control vehicle, linear PA or branched PA at their spinal cord injury site (week 0). Starting from the following week (week 1) to up to 3 months, the rats were assessed for their hind limb loco-mobility using standardized open field behavior scoring (BBB scoring) (Basso et al., 1996, Joshi and Fehlings, 2002b). During the scoring, the experimenters were blinded to the identity of the group to which the rats belonged (vehicle, linear PA or branched PA). After 12 weeks, we collected the BBB scores of each rat and quantitatively analyzed them. We found no functional improvement with either non-bioactive PA injected group compared with the vehicle control group, nor did we observe a difference between the two PA injected groups. This indicates that PA scaffolds capable of rendering stiffness support alone do not promote functional reclamation after spinal cord injury functional improvement in the absences of a bioactive epitope (such as IKVAV sequence). The findings also support the paper that IKVAV PA improves SCI recovery mainly through bioactive epitope (IKVAV) presentation.

DISCUSSION AND CONCLUSIONS

Advances in bioengineering and materials science have resulted in the creation of new materials that are promising candidates for therapeutic applications. Unlike conventional drugs which are typically soluble molecules, bioengineering materials that provide signals from an immobilized scaffold may benefit from their unique structural properties. In this paper we also demonstrated that two PAs with other bioactive epitopes (Tenascin C and RGD presenting PAs) exerted beneficial effects even larger than those of IKVAV-PA even though both of these PAs have the same stiffness structural support with IKVAV PA. This provides additional evidence that the bioactivity of the epitope is primarily responsible for the beneficial effects in spinal cord injury.

However, the conclusion that the stiffness property of the PA scaffolds wasn't the key factor for the therapeutic effects does not mean that the stiffness did not contribute. It is possible, and perhaps likely, that with a bioactive epitope attached the stiffness of scaffolds will contribute to the maximum presentation of the epitope. Thus modifying the stiffness of scaffold could possibly modify the accessibility of cells to contact and receive signal from the material, which ultimately could lead to a biological effect modification. This possibility can be addressed in future experiments that systematically examine changing the mechanical properties of epitope-presenting PAs.

This paper were designed to help advance knowledge about the pathophysiology of spinal cord injury and to explore new therapeutic options. At present there are no therapeutic interventions that enhance regeneration after spinal cord injury, and the only current treatments are targeted at limiting secondary damages.

There are multiple reasons for this. The first reason is the intrinsic vulnerability of central nervous tissue towards injury damage and the irreversibility of glial scar formation. The adult CNS has only limited regeneration capability. At the same time, the viability of neurons is critically dependent upon the delicate and integral microenvironment that the blood brain barrier helps to maintain. Once injuries have occurred and this balance is disrupted, sensitive neurons die, and a cascade of events is initiated that leads to a second wave of cell death in the early phases of spinal cord injury. Astrocytic hypertrophy helps to restore the blood brain barrier,

but a permanent glial scar is formed that acts as a physical barrier tor axonal regeneration.

Another impediment is insufficient understanding of the detailed pathophysiologic processes underlying SCI, which limits the development of novel therapeutic inventions. One potential approach to modify this process is to limit glial scar formation which should reduce a major hurdle for axon regeneration. Accomplishing this will require understanding the nature of the precursor cells that contribute to the scar, the signaling mechanisms that mediate the process of scar formation, and the precise nature of the cells that form the scar. Without such knowledge it will be difficult to develop a targeted strategy for therapeutic invention. The nature of the cells in the scar has been reasonably well characterized. The predominant cell type in the scar is the astrocyte along with other cells including microglia, endothelial cells and fibroblasts. Astrocytes are the key component that limits axon regeneration. The cell bodies of astrocytes not only form tight junctions and physically block axon regeneration but they also secrete and/or bind molecules that inhibit axon outgrowth. One major group of these molecules is chondroitin sulfate proteoglycan (CSPGs). CSPGs inhibit axon regeneration through epidermal growth factor receptor (EGFR) mediated pathways (Koprivica et al., 2005). Blocking CSPGs' inhibitory effect promotes axon regeneration after experimental spinal cord injury, achieving a therapeutic effect (Bradbury et al., 2002, Bukhari et al., 2011). This demonstrates in principle that recovery form SCI can be improved by limiting the detrimental effects of the glial scar.

Our group's approach to limiting the detrimental effects of glial scar formation is not only to limit the presence of growth inhibiting molecules but also to reduce the overall astrocyte cell population in the glial scar. This should not only reduce the detrimental effects of inhibitory molecules bound to astrocytes but also should help to alleviate the physical obstruction to axon outgrowth. This attempt to reduce the astrocyte cell population in the glial scar is backed by our understanding of the astrocyte compositions within the scar. Astrogliosis and scar formation is comprised of two waves of events.

The first phase could be summarized as local astrocyte hypertrophy. The injury induced toxic environment triggers local astrocyte reactive responses, with limited self-division and mainly cell body augmentation. This phase is considered beneficial for restoration of the blood brain barrier. The second and longer phase involves the influx of new astrocytes generated elsewhere that migrate into the injury site. These astrocytes add thickness to the scar tissue and stabilize it, but they are responsible for blocking axon regeneration. This recognition guides our attempt to mainly limit the second phase of astrocyte hyperplasia without limiting the initial hypertrophy.

To design intervention strategies for new astrocyte generation, understanding the process of post injury astrocyte generation is essential. This includes identification of the progenitor cell population(s) which give rise to astrocytes plus the signaling mechanisms that control this process. It has been over a decade that the field recognized the presence of multipotent neural stem cells in adult spinal cord, which are ependymal cells that surround the central canal (Weiss et al., 1996, Johansson et al., 1999). These cells have can give rise to neurospheres in vitro and are able to generate neurons, astrocytes and oligodendrocytes (Weiss et al., 1996, Johansson et al., 1999). Only recently was it recognized that the second wave of astrocytes generation following SCI actually came from ependymal cells (Meletis et al., 2008). Yet the signal mechanisms controlling astrocyte generation from ependymal cells are still largely unknown. This paper work has answered one piece of this question.

Guided from a previous observation that IKVAV PA altered astrogliosis post spinal cord injury (Tysseling-Mattiace et al., 2008), we initiated an in vitro lineage commitment study of the effects of IKVAV PA on neural stem cells. We found that the neural stem cell membrane receptor β1 integrin plays a key role in regulating NSC differentiation into astrocytes. Through further experiments of targeted alteration of gene expression, including virus mediated gene knockout and bioengineering based gene signal augmentation, we confirmed that β1 integrin signaling inhibits astrocytic lineage commitment by NSCs. In addition, we did signal transduction exploration downstream of β1 integrin and found that astrocytic repression by β1 integrin is mediated, at least in part, by integrin linked kinase (ILK). We were able to rescue the astrocytic suppressive effects in β1 integrin null NSCs by inducing kinase activated ILK expression. Furthermore, we were also able to observe an astrocyte differentiation alteration by merely modifying ILK's signal.

This in vitro mechanistic work guided our attention back to *in vivo* pathophysiology in a spinal cord injury animal model. We found activation of β1 integrin activation on NSCs in vivo in the IKVAV PA treated spinal cord, suggesting that the IKVAV PA reduced glial scar formation in vivo was achieved through activation of β1 integrin signaling. This suggests that targeted manipulation of β1 integrin signaling in acute stages of spinal cord injury might be a strategy for enhancing recovery. This is one very mechanistic hypothesis

that may guide and contribute to development of therapies for SCI therapy. Our hypothesis is supported by our recent finding that two additional PAs with epitopes known to activate β1 integrin (Tenascin C PA and RGDs PA) also showed beneficial effects in experimental SCI.

The SCI therapeutic mechanism we proposed above would only effective for acute SCI, because it is targeted to modify the astrogliosis that takes place within the first two to three weeks of injury. In order to treat chronic SCI, a different therapeutic strategy had to be explored. At chronic stages of spinal cord injury, glial scar progression has reached a static state. In order to promote regeneration, one attempt has focused breaking the blockade of glial scar and rebuilding axon's growth path. Different implantable nerve guidance channels have been extensively investigated (Straley et al., 2010, Joosten, 2012). In practice, the ideal nerve guidance channels should have following properties: non-toxic; degradable; resilient yet with sufficient mechanical strength for implantation; provide favorable environment for axons to grow, etc. Currently there is still no ideal material which can full fill all these criteria. What we report in this paper is a novel synthesized material with promising potential.

We showed that we are able to build a semi-automatic injection platform to inject a synthesized peptide solution into the spinal cord and create a nerve guidance channel capable of supporting neurite growth in vivo. This monodomain gel that we used is non-toxic, degradable, and mechanically strong enough to persist as an engraft. Moreover it also is able to provide bioactive signals through its epitope presenting capability and it guides neurite growth in a linear direction. This could potentially facilitate regrowth of fiber tracts such as the corticospinal tract. For a future research perspective, we plan to test this material's capability to guide endogenous axon regeneration, which hasn't been addressed in this paper.

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