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REVIEW

Neurotrophic factors in combinatorial approaches for spinal cord regeneration

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Abstract Axonal regeneration is inhibited by a plethora of different mechanisms in the adult central nervous system (CNS). While neurotrophic factors have been shown to stimulate axonal growth in numerous animal models of nervous system injury, a lack of suitable growth substrates, an insufficient activation of neuron-intrinsic regenerative programs, and extracellular inhibitors of regeneration limit the efficacy of neurotrophic factor delivery for anatomical and functional recovery after spinal cord injury. Thus, growth-stimulating factors will likely have to be combined with other treatment approaches to tap into the full potential of growth factor therapy for axonal regeneration. In addition, the temporal and spatial distribution of growth factors have to be tightly controlled to achieve biologically active concentrations, to allow for the chemotropic guidance of axons, and to prevent adverse effects related to the widespread distribution of neurotrophic factors. Here, we will review the rationale for combinatorial treatments in axonal regeneration and summarize some recent progress in promoting axonal regeneration in the injured CNS using such approaches.

Keywords Neurotrophin · Gene therapy · Axonal regeneration · Spinal cord injury · Cell transplantation

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Introduction

In vitro and in vivo studies conducted over the last 30 years have identified several key issues that need to be considered to improve axonal regeneration in the adult mammalian central nervous system (CNS) (Table 1). There is ample evidence that axon growth-inhibitory molecules present in the adult injured CNS contribute to an unfavorable environment for axonal regeneration (Silver and Miller 2004; Xie and Zheng 2008). Glial and inflammatory reactions after injury further add to the adverse milieu of the injured spinal cord and diminish the prospects for axonal regeneration (Bethea and Dietrich 2002; Fitch and Silver 2008; Popovich and McTigue 2009). Molecules and guidance cues present in the developing nervous system are not preserved throughout adulthood or have different functions, resulting in an insufficient stimulation of axonal growth and limited means to direct axons towards appropriate targets (Giger et al. 2010). Neuronal and glial cell death at a site of injury cannot be easily replenished from the intrinsic neural stem cell pool, and cystic degeneration resulting in the formation of fluid-filled cavities at sites of spinal cord injury (SCI) requires the provision of a substrate for axons to bridge across a lesion site. Finally, differentiated neurons in the adult CNS do not activate transcriptional programs that are adequate and sufficiently sustained to allow for regeneration to occur.

No single experimental treatment can tackle all of these factors and will therefore be limited in its efficacy to augment axonal regeneration. During development and axon regeneration in the injured peripheral nervous system (PNS), multiple mechanisms interact to contribute to efficient and controlled axonal growth. Physical guidance by appropriate cellular orientation and provision of a growth-permissive extracellular matrix clearly contribute to directed

Table 1 Selected combinatorial therapies for axonal regeneration after experimental spinal cord injury

Axonal population	Cell soma stimulation	Substrate at the lesion site	Neutralization of inhibition	Growth factor	References
Serotonergic, noradrenergic, and corticospinal axons	-	E14 Fetal graft	-	BDNF +/− NT-3, NT-4 protein NT-3/BDNF infusion	Bregman et al. 1993 Coumans et al. 2001
Serotonergic propriospinal, vestibulospinal, reticulospinal, raphaespinal, and other bulbospinal axons	-	Schwann cells + olfactory ensheathing cells	ChABC	-	Fouad et al. 2005; Vavrek et al. 2006
Serotonergic axons	cAMP + rolipram	Schwann cells	cAMP + Rolipram	-	Pearse et al. 2004 but see Sharp et al. 2010
Dorsal column sensory axons	cAMP	Bone marrow stromal cells	-	NT-3 protein	Lu et al. 2004
Dorsal column sensory axons	Conditioning lesion	Bone marrow stromal cells	-	NT-3 lentivirus	Alto et al. 2009; Blesch et al. 2012; Kadoya et al. 2009
Reticulospinal, raphaespinal, rubrospinal, coeruleospinal, and propriospinal axons	-	Peripheral nerve graft	ChABC	-	Houle et al. 2006
Descending axons	-	Peripheral nerve graft	ChABC	GDNF infusion	Tom et al. 2009
Corticospinal and serotonergic axons	-	Neural stem cells	ChABC	EGF + bFGF + PDGF-AA infusion	Karimi-Abdolrezaee et al. 2010
Serotonergic axons	Rolipram (cAMP)	Fetal graft	Rolipram	-	Nikulina et al. 2004
Transplanted DRG neurons	-		ChABC	NT-3 lentivirus	Massey et al. 2008
Serotonergic axons	-	-	Thermo-stabilized ChABC/trehalose	NT-3 protein	Lee et al. 2010
Rubrospinal axons	LiCl-induced suppression of GSK-3β/ upregulation of Bcl-2	-	ChABC	-	Yick et al. 2004
Serotonergic and descending bulbospinal axons	-	Peripheral nerve graft	ChABC	-	Alilain et al. 2011

axonal extension. In both circumstances, neurons have an intrinsically high capacity for axonal growth based either on their developmental status (Blackmore et al. 2010; Moore et al. 2009) or on the initiation of appropriate changes in gene expression (Michaelevski et al. 2010). At the axon tip, growth is also stimulated by levels of growth factors higher than those present in the adult CNS, and expression patterns of growth factors are tightly regulated in a spatial and temporal manner. Thus, in both situations, multiple mechanisms act synergistically to result in significant axonal growth. The above-mentioned examples from development and PNS injury, and the empirical evidence about limited effects of single treatments on axonal regeneration in the injured spinal cord, corroborate a need to address more than one factor in axon regeneration failure. In this review, we aim to summarize recent data on regeneration, sprouting, and plasticity of injured axons following SCI. We will focus on studies that have combined means to stimulate axonal growth, including neurotrophic factor delivery, cellular grafts, neutralization or digestion of growth-inhibitory molecules, and activation of the intrinsic growth capacity of injured neurons.

Bridging the lesion site

The significance of the environment for adult CNS regeneration became for the first time clearly evident in a landmark study by Aguayo and colleagues, showing that brainstem and spinal cord neurons can extend axons into an implanted peripheral nerve graft (David and Aguayo 1981; Richardson et al. 1980). At the same time, these studies also demonstrated

that adult neurons in the CNS retain some capacity for regeneration if they are provided with a favorable milieu, challenging earlier beliefs that these neurons completely lack the ability to activate any intrinsic regenerative program.

These studies stimulated the search for a cellular or acellular matrix that could mimic some aspects of a peripheral nerve graft or provide an even superior environment. Cellular grafts comprised of fibroblasts, bone marrow stromal cells (Ankeny et al. 2004; Hofstetter et al. 2002), Schwann cells (Li and Raisman 1994; Tuszynski et al. 1998; Weidner et al. 1999; Xu et al. 1997, 1995, 1994), olfactory ensheathing cells (Li et al. 1997; Lu et al. 2006; Ramon-Cueto et al. 2000; Ramon-Cueto and Nieto-Sampedro 1994; Richter and Roskams 2008), stem cells (Lepore and Fischer 2005; Lu et al. 2003; Pfeifer et al. 2004), and embryonic tissue (Bregman et al. 1993; Mori et al. 1997; Stokes and Reier 1992; Tessler 1991; Tessler et al. 1997) are some of the cellular substrates that have been examined in this context (Tetzlaff et al. 2011). However, a uniform cellular graft lacks important aspects found in the regenerating PNS. Upon injury in the PNS, profound changes in the expression patterns of neurotrophic factors can be observed, changes which have proven critical to successful peripheral nerve regeneration by studies using knockout animals and neutralization of growth factors (English et al. 2005; Geremia et al. 2010; Zhang et al. 2000; Zhong et al. 1999). Schwann cells upregulate expression of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), and other growth factors in particular in the distal nerve stump, resulting in chemotropic growth factor gradients. Upon reinnervation of the distal nerve, expression of growth factors declines (Funakoshi et al. 1993; Heumann et al. 1987; Naveilhan et al. 1997; Sendtner et al. 1992; Trupp et al. 1997). When grafted into the injured spinal cord, available data support that a peripheral nerve may retain patterns of neurotrophic factor upregulation observed after PNS injury, whereby NGF levels exhibit two peaks, immediately at the time of injury and some days following the injury, while BDNF levels gradually increase and peak at later time points (Kuo et al. 2011; Meyer et al. 1992). Thus, a regulated spatial and temporal expression pattern of growth factors contributes to effective and rapid regeneration in the PNS.

Studies combining cell transplantation with neurotrophic factors using genetically modified cells have aimed to mimic some components of the growth-promoting environment of the PNS. Indeed, robust axonal growth into cellular transplants can be induced by neurotrophic factor production from grafts of genetically engineered cells. In summary, NGF and GDNF can induce growth of peptidergic and non-peptidergic nociceptive axons, respectively (Blesch and Tuszynski 2003; Tuszynski et al. 1996), growth of reticulospinal, raphaespinal, rubrospinal and propriospinal axons is augmented by BDNF and NT-4/5 (Blesch et al. 2004; Liu et al. 1999; Lu et al. 2005; Menei et al. 1998), and NT-3 enhances growth of ascending sensory axons (Bradbury et al. 1999; Taylor et al. 2006). NT-3 delivery in the injured spinal cord also induces sprouting of corticospinal axons (Blits et al. 2000; Fortun et al. 2009; Grill et al. 1997; Schnell et al. 1994; Zhou et al. 2003). While studies on cellular growth factor delivery indicated substantial growth responses for many axonal populations into the lesion site, corticospinal axons failed to penetrate most cellular grafts examined to date. Instead, corticospinal axon sprouting was mainly observed in remaining gray matter in partial lesion models. Given the importance of corticospinal projections in fine motor control in humans and primates, the quest for an appropriate substrate for the corticospinal tract therefore remains a high priority.

Equally importantly, axons responding to cellular delivery of neurotrophic factors rarely exit cellular grafts to regenerate across the lesion site, limiting the usefulness of this approach. Only when growth factors are delivered beyond the lesion site in combination with cell or tissue transplantation have regenerating axons been shown to bridge across a site of SCI (see below).

Guidance and directional growth of regenerating axons

After injury in the PNS, a regulated spatial and temporal expression pattern of neurotrophic factors contributes to successful regeneration through context-dependent survival, growth, and guidance signaling. Numerous studies have described the neurotrophin family, consisting of NGF, BDNF, NT-3, and NT-4/5, and their tyrosine kinase receptors TrkA, -B, and -C and the low-affinity p75 neurotrophin receptor, which are integral to regenerative processes (Boyd and Gordon 2003; Cui 2006). Upon neurotrophin binding to their appropriate Trk receptor along the axon, the activated complex is endocytosed and retrogradely transported via microtubule/dynein machinery to the cell soma to propagate signaling cascades that underlie neuronal survival and growth (Neet and Campenot 2001). In addition to promoting neuronal survival, neurotrophins are also strong stimulators of axonal growth, specifically when receptors in the axon tip are activated (Campenot 1982; Kimpinski et al. 1997; Zhou and Snider 2006). Neurotrophin stimulation at the growth cone can also induce chemotropic guidance, in vitro (Gundersen and Barrett 1979) and in vivo in the PNS (Hu et al. 2010) and CNS (Alto et al. 2009; Taylor et al. 2006).

Assuming that injured axons in the CNS are as responsive to growth factor gradients, as they are during PNS regeneration, delivery of growth factors only into the SCI lesion site may actually prevent axons from exiting the

lesion site and reentering the distal host spinal cord. Indeed, studies examining growth factor-expressing cellular grafts have rarely reported axon growth across the lesion site. Only if neurotrophin delivery is modified such that a growth factor gradient is established with highest concentrations beyond the lesion site, can axons bridge a site of SCI and reenter the host spinal cord. This was demonstrated using a combination of bone marrow stromal cell grafts at the lesion site and lentiviral NT-3 gene transfer distal to a spinal cord lesion site, allowing for ascending dorsal column sensory axons to extend across a lesion site (Taylor et al. 2006). In contrast, delivery of NT-3 only within the lesion leads to growth into but not beyond a lesion in the injured spinal cord (Lu et al. 2003). Similarly, transplanted dorsal root ganglion (DRG) neurons can cross a lesion in the corpus callosum towards a viral NGF source (Jin et al. 2008). Regeneration and guidance of sensory axons across the dorsal root entry zone has also been demonstrated towards areas of highest neurotrophin expression (Romero et al. 2000). These responses can be further refined by combining chemoattractive and chemorepulsive guidance cues, thereby restricting the reinnervation pattern of sensory axons in the spinal cord or directing the growth of transplanted DRG neurons (Tang et al. 2004; Ziemba et al. 2008). Taken together, these studies support the hypothesis that injured adult neurons can respond to growth-promoting and growth-inhibiting guidance factors during axonal regeneration. By combining appropriate substrates with gradients of attractive and repulsive cues, more directed axonal growth can be achieved.

Directional growth of axons has also been demonstrated for grafts of neural precursors. Upon grafting a mixture of embryonic neural- and glial-restricted precursors into a spinal cord lesion site, axons originating from grafted neurons extend preferentially towards a source of BDNF established by lentiviral gene transfer (Bonner et al. 2010). If progenitor grafts are close to the dorsal column nuclei, they can form synapses and serve as a relay for injured dorsal column sensory axons (Bonner et al. 2011).

In addition to an appropriate spatial distribution of growth factors, the temporal availability of axon growth-promoting stimuli might be equally important. Cellular growth factor delivery in a lesion site has the potential to increase the pool of axons available to extend from a lesion into the distal spinal cord, but continuous expression in a lesion site certainly does not promote axonal growth into the inhibitory environment present beyond the lesion site. On the contrary, long-term overexpression of neurotrophic factors by genetically modified cells can lead to a gradual increase in graft size at least partially due to the invasion and proliferation of Schwann cells (Blesch and Tuszynski 2003; Blesch et al. 2004). Experiments with regulated neurotrophic factor expression further indicate that high levels of trophic factors are only necessary to induce growth into a lesion, whereas low levels are sufficient to sustain axons that have extended into a cellular graft (Blesch and Tuszynski 2007). Clearly, there is an urgent need for efficient and safe means to regulate neurotrophic factor expression for axonal regeneration in vivo or, alternatively, vectors that automatically turn off within a defined time period due to epigenetic silencing.

Approaches to reproduce the physical guidance properties found in the PNS have also been described in recent years using different forms of biomaterials. Cellular grafts lacking orientation and alignment of grafted cells along the longitudinal axis of the spinal cord result in randomly directed axonal growth, thereby effectively limiting the size of a lesion that axons can successfully bridge. This is of particular importance when translating findings to the injured human spinal cord. The larger extent of lesions in the injured human spinal cord will require axons to bridge over significantly longer distances than any rodent studies have shown to date. Increasing the distance and rostro-caudal orientation of axonal growth therefore remains an important objective for translating axonal regeneration in the foreseeable future. Using linear scaffolds with defined pore sizes that can be filled with a cellular substrate, a more directional, linear axonal growth pattern can be achieved (Prang et al. 2006; Stokols et al. 2006; Straley et al. 2010). When combined with neurotrophin gene transfer, the vast majority of axons entering a scaffold can extend throughout this bridge and are found to exit distally (Gros et al. 2010). The latter study also activated a regenerative program in injured neurons, as discussed below.

Activating cell body responses to injury

Upon injury in a distal axon segment, a retrograde signal travels for extended distances (possibly more than 1 m), manifesting in responses in injured neuronal cell bodies (Hanz et al. 2003). This response has marked influence on the success or failure of axon regeneration. It can involve substantial, long-term changes in the expression of thousands of genes until target innervation is achieved (Costigan et al. 2002; Goldberg et al. 2002; Michaelevski et al. 2010; Stam et al. 2007; Xiao et al. 2002) or it can lead to cellular atrophy (Kwon et al. 2002), or, in the worst case, to neuronal cell death (Giehl and Tetzlaff 1996). The latter was initially thought to be prominent in corticospinal motor neurons after SCI. However, more recent studies have indicated that corticospinal neurons do not die but become atrophic (Brock et al. 2010; Nielson et al. 2011), a reaction that has also been observed for neurons in the red nucleus following spinal cord lesions (Kobayashi et al. 1997).

Preventing neuronal atrophy and activating regenerative programs in injured neurons might be a prerequisite for

long-distance axonal regeneration. One of the most characterized systems for the activation of regenerative programs in injured neurons is the sensory system and so-called conditioning lesions (Richardson and Issa 1984). If the central branch of dorsal column sensory axons is injured in the spinal cord, regeneration and sprouting does not occur. However, if a lesion of the peripheral branch precedes lesions of the central branch, neurons are pre-conditioned, and axons activate a regenerative program. This results in sprouting into or around a spinal cord lesion site or into a peripheral nerve graft (Neumann and Woolf 1999; Richardson et al. 1984). The signaling cascades leading to the activation of this regenerative program remain incompletely understood, but increases in intracellular second messengers, modulation of cytoskeletal signaling, and upregulation of cytokines and growth factors have been implicated in this response. In particular, elevation of cAMP (Cai et al. 2001; Gao et al. 2004; Hannila and Filbin 2008; Lu et al. 2004; Neumann et al. 2002; Qiu et al. 2002), suppression of Rho signaling or constitutively activated growth cone components (Dergham et al. 2002; Jain et al. 2011), and activation of stat3 via neuropoetic cytokines (IL-6/LIF/CNTF) (Cafferty et al. 2004; Cao et al. 2006; Qiu et al. 2005; Wu et al. 2006) seem to play major roles in enhancing growth in an inhibitory CNS environment. Whereas a conditioning lesion preceding a central lesion has been clearly shown to enhance localized sprouting at a lesion site, a more significant effect is apparent when combined with neurotrophic factors to stimulate axonal growth. Provision of bone marrow stromal cells in a dorsal column lesion site, combined with lentiviral NT-3 gene transfer rostral to the lesion site and a conditioning lesion, increases the number and distance of sensory axons bridging across the lesion site (Alto et al. 2009). Enhanced growth of sensory axons in such a combination is also observed if preconditioning lesions are replaced by elevations of cAMP (Lu et al. 2004), although it is likely that cAMP is only partially responsible for effects observed after conditioning lesions. Importantly, even if peripheral neurons are conditioned after the central lesion in a chronic model of SCI, sensory axons extend across the grafted cellular bridge into the distal host spinal cord (Blesch et al. 2011; Kadoya et al. 2009). Although the in vivo regenerative response is diminished compared to an acute lesion (Blesch et al. 2011), in vitro neurite growth assays and changes in DRG gene expression suggest that the intrinsic growth program is equally activated.

Much less is known about the activation of regenerative programs in CNS neurons. One of the best-studied CNS systems in regeneration is that of retinal ganglion neurons after optic nerve injury. Lens injury activates a signaling cascade that enhances optic nerve regeneration, and the mechanisms underlying this cascade have been partially identified. Activation of stat3 via CNTF/LIF appears to be one major factor (Muller et al. 2009). In addition, PTEN knockout and siRNA studies in mice suggest that the activation of PI3-kinase and the mTOR pathway can substantially enhance optic nerve regeneration (Park et al. 2008). If activation of PI3-kinase via PTEN knockout is combined with activation of stat3 via SOCS3 knockout, the number and distance of regenerating axons are even further enhanced (Sun et al. 2011). Activation of the mTOR pathway can also enhance corticospinal sprouting in mice, at least if it is activated prior to SCI (Liu et al. 2010). As both pathways have been shown to contribute to the conditioning lesion effect in sensory axon regeneration, it appears that general principles in the activation of intrinsic regenerative programs exist.

Practical means to activate growth programs in injured CNS neurons with projections to the spinal cord are limited, and only a few have been identified to date. Infusions and viral gene delivery of neurotrophins have been examined for several neuronal populations. In the red nucleus, provision of BDNF at the cell soma has been shown to ameliorate neuronal atrophy even at chronic time points up to 1 year post-lesion (Kwon et al. 2007; Kwon et al. 2002; Ruitenberg et al. 2004). In addition, BDNF also increases expression of regeneration-associated genes, like alpha-tubulin and GAP43 (Kobayashi et al. 1997), promoting growth of rubrospinal axons into peripheral nerve grafts placed in the lesioned spinal cord (Kobayashi et al. 1997; Kwon et al. 2002).

Infusion of BDNF into the motor cortex can apparently also activate regenerative responses, evident in enhanced corticospinal collateral sprouting in the injured spinal cord (Vavrek et al. 2006). Although BDNF can reverse corticospinal neuronal atrophy in rodents and primates even when provided in a spinal cord lesion site (Brock et al. 2010), BDNF or NT-4/5 delivered by genetically modified fibroblasts in the spinal cord fail to increase corticospinal sprouting after a midthoracic injury (Blesch et al. 2004; Lu et al. 2001). Indeed, BDNF delivery to corticospinal axons appears to only enhance regeneration into a transplant when the cognate receptor, trkB, is overexpresssed by corticospinal neurons and BDNF-expressing grafts are placed in a subcortical lesion site (Hollis et al. 2009).

Other studies have focused on genes important in CNS development to enhance the innate regenerative capacity (Blackmore et al. 2010; Moore et al. 2009; Wong et al. 2006; Yip et al. 2006, 2010) or cell adhesion molecules (Andrews et al. 2009), but these treatments have not been combined with substrates for axonal growth or other means to stimulate axon regeneration.

Target innervation and functional integration

If axons regenerate across a lesion in the injured spinal cord, finding appropriate targets and establishing functional synapses will be a prerequisite for behavioral recovery. From

hundreds of neurons present in the vicinity of a regenerated axon, proper connections need to be formed to have functional implications. Spontaneous recovery of function after SCI can at least partially be attributed to localized axon sprouting and the formation of new connections. For example, corticospinal and reticulospinal axons have been shown to sprout towards propriospinal neurons and motor neurons after SCI (Ballermann and Fouad 2006; Bareyre et al. 2004; Fouad et al. 2001; Girgis et al. 2007; Vavrek et al. 2006; Weidner et al. 2001; Z'Graggen et al. 2000). Extensive collateral sprouting that is accompanied by behavioral recovery is also observed in the primate spinal cord (Rosenzweig et al. 2010). Propriospinal neurons can serve as relays to allow for supraspinal control of motor function without any direct connections beyond a lesion in the spinal cord (Courtine et al. 2008).

Taken together, these studies indicate that some natural mechanisms exist that support the formation of appropriate new connections after SCI. However, rather little is known about the molecular basis and the guiding principles of these changes. Use- and disuse-related Hebbian mechanisms might be at least partially involved in these processes. Recent data suggest that inherent mechanisms of sprouting and synapse formation might be strengthened by rehabilitative training. Rehabilitation and training, possibly via upregulation of neurotrophic factors including BDNF and insulin-like growth factor 1 by specific neuronal populations, could provide one means to attract or stabilize synaptic connections (Gomez-Pinilla et al. 2002; Hutchinson et al. 2004; Vaynman and Gomez-Pinilla 2005; Ying et al. 2005).

Combining neurotrophins with degradation of inhibitory molecules

Inhibitory extracellular matrix including chondroitinsulfate proteoglycans (CSPGs) is one key factor limiting axonal regeneration in the injured mammalian CNS. Within 24 h post-injury, reactive astrocytes begin to deposit high amounts of CSPGs at the lesion site, establishing a long-lasting inhibitory boundary that has been comprehensively shown in vitro and in vivo to hinder regenerative growth of adult CNS axons and to inhibit synaptogenesis (Jones et al. 2003; Silver and Miller 2004). Degradation of the glycosaminoglycan (GAC) side chains of CSPG molecules by local administration of the enzyme chondroitinase ABC (ChABC) can enhance local sprouting and facilitate axonal regeneration, in part by reducing the inhibitory components of the glial scar and disrupting the CSPG perineuronal nets which envelope neuronal somata (Bartus et al. 2011; Galtrey et al. 2008). Without a cellular graft or trophic support, ChABC treatment upon acute or subchronic SCI induces moderate plasticity of surrounding tissue and some regeneration of select axonal tracts (Alilain et al. 2011; Bradbury et al. 2002), even when coupled with acute or delayed rehabilitative training (Garcia-Alias et al. 2009; Wang et al. 2011) or a systemic pharmacological neuroprotective agent (Yick et al. 2004). When administered in parallel with a graft at the lesion site, ChABC has been shown to increase the number of axons regenerating across the lesion site into the distal host spinal cord (Chau et al. 2004; Fouad et al. 2005; Houle et al. 2006; Tom and Houle 2008). In combination with GDNF delivered directly to and around the lesion site to activate regenerative responses in chronically injured neurons, ChABC and a peripheral nerve graft applied 4 weeks post-injury stimulates descending axons to regenerate into and through a PNS graft and exit to form new connections (Tom et al. 2009). The fast rate of inactivation of ChABC in vivo at physiological temperatures might be one limiting factor in ChABC-mediated effects on morphological and functional recovery. A recently described thermostabilized form of ChABC (ChABC/trehalose) results in suppressed CS-CAG levels for 6 weeks after SCI, threefold longer than previously shown (Lee et al. 2010). When ChABC/trehalose was employed in parallel with NT-3 in an agarose gel scaffold in the lesion site, a significant increase of serotonergic axon sprouting was observed adjacent to the lesion compared to control groups. Using a similar combination of ChABC (microinjection) and NT-3 (lentiviral delivery), the growth of transplanted adult DRG neurons into the CSPG-rich dorsal column nuclei can be increased 10-fold compared to ChABC or NT-3 treatment alone, strongly suggesting a cooperative interaction between mechanisms of disinhibition and a boost in growth-promoting signal (Massey et al. 2008). Of clinical relevance, several studies consistently demonstrate that pathways facilitating pain perception appear not to be influenced by ChABC treatment (Barritt et al. 2006; Karimi-Abdolrezaee et al. 2010). Taken together, removal of inhibitory extracellular matrix components in conjunction with neurotrophin delivery appears to synergistically enhance axonal regeneration.

Conclusions

Over the last two decades, it has become increasingly clear that the lack of axonal regeneration in the mammalian CNS is not due to a single inhibitory mechanism or entirely to the reticence of CNS axons to regenerate. The complexity of SCI and the numerous factors influencing regeneration provide a clear rationale for combinatorial treatments, and several studies have now successfully demonstrated that effects of neurotrophic factors can be further increased if growth factors are combined with other treatments, and vice versa. However, in vivo experiments examining multiple treatment parameters are laborious and demanding, requiring a large number of controls to test each single treatment

and possible combination. A regressive analysis with control groups that receive systematically fewer treatments might be one means to collect evidence for improvements without initial testing of all necessary controls. In addition, interactions between treatments could not only change the dose response profile of each therapy but also lead to negative consequences and functional deterioration. A better understanding of the mechanisms of each single treatment and more localized targeting of therapeutic approaches might be helpful to avoid adverse outcomes. Numerous challenges remain, but empirical evidence from several experimental treatments suggests that improved outcomes are possible with combinatorial strategies, justifying the further pursuit of multi-mechanistic approaches.

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