Review

Advances of exogenous agents to treat spinal cord injury

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Abstract Abstract

Treatment of spinal cord injury seemed frustrating and hopeless because of the remarkable morbidity and mortality, and restricted therapeutic options. Recent advances in neural injury and repair, development of neuroprotective and regenerative interventions are basis for increased optimism. Spinal cord injury is a debilitating condition. Therefore, amelioration of the microenvironment in the injured spinal cord becomes crucial for its recovery. A number of potential approaches aim to optimize functional recovery after spinal cord injury have been studied, including minimizing the progression of secondary injury, manipulating the neuro-inhibitory environment of the spinal cord, replacing lost tissue with transplanted cells or peripheral nerve grafts, remyelinating denuded axons, and maximizing the intrinsic regenerative potential of endogenous progenitor cells. In this review, we mainly focused on the exogenous agents used in these studies of spinal cord injury, and hope to summarize some valuable information for further studies and treatments of clinical cases

Key words: *Spinal cord injury; Stem cells; Virus vector; Inhibitor*

Introduction

The regeneration and functional recovery of neurons have been a bottleneck problem to solve spinal cord injury in clinical cases. Besides, through exploring the endogenous mechanism of the recovery, scientists have tried to find out the possible treatment through exogenous agents. This paper reviewed the latest literatures, focused on the latest development in this field and tried to give some suggestion for future exogenous research in spinal cord injury.

We will talk about the subject in some aspects as below: 1) cell-based approaches; 2) methods using Virus-based vectors; 3) inhibitory interventions; 4) combined methods; 5) Therapeutic electrical stimulation.

Cell-based approaches

Basic science advances in spinal cord injury and regeneration research have led to a variety of novel experimental therapeutics designed to promote axonal regrowth and sprouting. Some of these interventions belong to cell-based approaches which have potential for restoring damaged neural pathways and reconstructing intraspinal synaptic circuitries by either regeneration or neuronal/glial replacement (Ormond D R, et al., 2014).

A variety of adult stem cells have been implanted in rat models of spinal cord injury, ranging from olfactory ensheathing cells, cultured spinal cord stem cells, bone marrow derived stem cells to dermis derived stem cells (Majczynski H, et al., 2005). Recent progress in the stem cell biology has led much insight into the new therapeutic interventions aiming for the regeneration of the damaged central nervous system. The major strategies of cell transplantation can be classified into two subgroups i.e. activation of endogenous neural stem cells and cell transplantation therapies. In this review, we mainly focused on the cell transplantation therapies.

Bone marrow stromal cells

Bone marrow stromal cells (BMSCs) have great potential as therapeutic agents since they are easy to isolate and can be auto-transplanted without serious ethical and technical problems.

BMSCs were considered to be suitable cell types for ex-vivo gene delivery and able to support host axonal growth after spinal cord injury. Combination with other experimental approaches was required to achieve axonal growth beyond the lesion site and functional recovery (Bertram J P, et al., 2010).

Some studies infused MSCs into the cerebrospinal fluid of contused rat to further investigate their effects on functional recovery, lesion morphology and axonal growth. Results showed that the BBB score was higher, and the cavity volume was smaller in rats with transplantation than in the control rats. Considering that MSCs can be used for autologous transplantation, it may impose a minimal burden on patients(Ankeny DP, et al., 2004; Ohta M, et al., 2004) .

Satake et al investigated the survival and migration of transplanted mesenchymal stem cells (MSCs) through the subarachnoid space into complete injured thoracic spinal cord tissue following injection into the caudal lumbar spine. They found that transplanted MSCs can migrate to

the injured thoracic spinal cord. Meanwhile, some MSCs differentiated into nestin-positive immature neurons and glial cells (Satake K, et al., 2004). And cells exerted effects by producing some neurotrophic factors into the CSF or contacting with host spinal tissues to reduce the cavities and improve the behavioral function in the rats with spinal cord injury (Ohta M, et al., 2004)

Some other studies focused on the human mesenchymal stem cells (HMSCs) and reported that HMSCs have no immunocompetent and could trespass species defense barriers. Animals treated with these cells have a better outcome than controls. Therefore, they propose that universal HMSCs from donors could be cultured, expanded and cryopreserved for further using in human organs regeneration (Mansilla E, et al., 2005).

Results from reports above supported that further investigation of MSCs is needed for the promising clinical treatment of spinal cord injury. MSCs can be used for autologous transplantation and the CSF infusion of transplants impose a minimal burden on patients.

Embryonic stem cells

Current concepts of the pathophysiology, repair and restoration of function in the damaged spinal cord are presented with an overlay of how neural stem cells, particularly ES cells, fit into the picture as important scientific tools and therapeutic targets.

Majczynski et al reported the improvements in hind limb locomotor-like movements following grafting of embryonic raphe nuclei cells into the spinal cord below the level of total transection in adult rats. And they also showed that the graft-induced restitution of hind limb locomotor is mainly caused by the new serotonergic innervation of the host spinal cord circuitry from the grafted neurons, being mediated by 5-HT2 receptors (Majczynski H, et al., 2005).

To further investigate the effects of L1 in spinal cord injury, Chen et al transfected embryonic stem cells with a plasmid encoding the full-length mouse L1 molecule under the control of PGK promoter. And their observations showed the outcome of neuritis in the transfected group was better than the controls. These results encourage the usage of L1-transfected embryonic stem cells which express L1 not only at the cell surface, but also as a soluble and secreted form. This method could condition the inhibitory environment for homophilic L1-enhanced axon regrowth not only in spinal cord regeneration, but also in other lesion paradigms (Chen J, et al., 2005).

Though there were many literatures reporting the beneficial contribution of ES cells transplantation in the recovery of spinal cord injury, some evidences still showed that they might be harmful to the spinal cord injury. Murine embryonic stem cells were induced to differentiate into neural lineage cells by exposing to retinoic acid. The moderate contusion injured rats received transplantation of cells genetically modified to over-express bcl-2 or the wild-type ES cells from which the bcl-2 line was developed. The results suggested that transplanting KD3 ES cells, or apoptosis-resistant cells derived from the KD3 line, into the injured spinal cord does not improve locomotor recovery and can lead to tumor-like growth of cells, accompanied by increased debilitation, morbidity and mortality (Howard MJ, et al., 2005).

Neural progenitor cells

Neural progenitor cells, including neural stem cells (NSCs), are an important potential graft material for cell therapeutics of damaged spinal cord.

Nakamura et al found transplantation of embryonic spinal cord-derived neurospheres supported the growth of supraspinal projections and functional recovery after spinal cord injury in the neonatal rat (Nakamura M, et al., 2005). And they also used a NSC-enriched population derived from human fetal spinal cord and expanded in vitro by neurosphere formation. NSCs labeled with BrdU (TP) were transplanted into the adult marmoset spinal cord after contusion injury. Their results indicated that in-vitro expanded NSCs derived from human fetal spinal cord are useful sources for the therapeutics of spinal cord injury in primates (Nakamura M, et al., 2005).

Another study also gave a similar results, which indicated that hCNS-SCns may possess therapeutic potential for CNS injury (Cummings BJ, et al., 2005).

Some evidences indicated that neurosphere-forming cells from human adult olfactory epithelium could be considered as an autologous source of stem cells for spinal cord repair (Alves F R, et al., 2010). The forebrain-derived neural precursor cells (NSPCs) could be used as an alternative of spinal-cord-derived NSPCs as a potential therapeutic agent for spinal cord injury (Shah S K, et al., 2011).

Since it has minimally invasive, intrathecal delivery of NPCs at lumbar spinal cord (lumbar puncture) represents an important and clinically applicable strategy. Lepore et al examined whether NPCs can be delivered to the hemisection injured cervical spinal cord via lumbar puncture using a mixed population of neuronal-restricted precursors (NRPs) and glial-restricted precursors (GRPs). They found that the grafts could be efficiently delivered to the lesion site and survival at least 5 week there. Moreover, there were few graft-derived cells localized to areas outside the injury site. So it is possible to deliver lineage-restricted NPCs using the minimally invasive lumbar puncture method for the treatment of spinal cord injury (Lepore AC, et al., 2005).

Fujiwara et al transplanted NPCs intravenously into each animal 24h after contusion injury. It was found that the injected NPCs migrated to the lesion site widely and showed nestin at an early phase after transplantation. These NPCs differentiated into neurons, astrocytes and oligodendrocytes, and survived at least for 56 days. These results indicated that intravenously injected neural stem cells could migrate into the spinal cord lesion meanwhile preserving their potential as NPCs, and that this procedure is a potential method of delivering cells into the lesion for the treatment of spinal cord injury (Fujiwara Y, et al., 2004).

Scientists found that the grafted human NSPCs survived and differentiated into neurons, astrocytes and oligodendrocytes, and that the cavities were smaller than those in sham-operated control animals at eight weeks after transplantation of NSPCs in cervical contusion rats. Therefore, NSPCs

transplantation was effective for SCI in primates and suggested that human NSPC transplantation could be a feasible treatment for human SCI (Iwanami A, et al., 2005). After cells of the murine NSCs were grafted into the acutely injured spinal cord of rat, they could survive and undergo partial differentiation. Notably, NSCs induced the de novo formation of host axon tracts aiming at graft innervation. These results showed that NSCs might not only play a critical supportive role in repairing axonal injury in the adult spinal cord, but also be used as probes for exploring the molecular underpinnings of the regenerative potential of the mature nervous system after injury(Yan J, et al., 2004; Pfeifer K, et al., 2004) . Evidences showed that the tropic/ trophic interactions of these stem cells with host axons, and the ensheathing properties of these cells were related to their complex molecular profile, which includes the expression of trophic cytokines and neurotrophins such as glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor, glial growth factor receptors (ErbB-2) and PASK, the mammalian homologue of the fray gene that is involved in axon ensheathment(Yan J, et al., 2004; Pfeifer K, et al., 2004) .

From previous reports we can know that there are at least three methods to transplant NPCs i.e. immediate transplantation of the stem cells into the injury site, intrathecal delivery at lumbar spinal cord (lumbar puncture) and intravenous injection. And all the methods have been successfully identified in the experiments.

Though many studies reported functional improvement after transplantation of neural stem cells into the injured spinal cord, Hofstetter et al provided evidence that grafting of adult neural stem cells into a rat thoracic spinal cord weight-drop injury improves motor recovery but also causes aberrant axonal sprouting associated with allodynia-like hypersensitivity of forepaws (Sumaira Pervaiz S I, 2012).

Glia cells

Several kinds of glia cells have been used into the studies of spinal cord injury, including Schwann cells, fibroblasts, oligodendrocyte and Olfactory ensheathing cells.Keyvan-Fouladi et al reported that transplantation of cultured adult peripheral nerve Schwann cells restored function, but the effect is delayed until 30 days after transplantation and reaches only 5-10% of normal (Keyvan-Fouladi N, et al., 2005).Mitsui et al found that the fibroblasts, genetically modified to express BDNF and NT-3 (Fb-BDNF/NT3) and transplanted into a thoracic spinal injury site, would enhance the recovery of bladder function, indicating that this treatment would be associated with reorganization of lumbosacral spinal circuits implicated in bladder function (Mitsui T, et al., 2005).

Recently, oligodendrocyte-type 2 astrocyte (O-2A) progenitor cells have been reported to remyelinate focal areas of demyelinated axons of spinal cord in adult rats. Lee et al investigated the therapeutic potential of transplantation of O-2A cells in a rat model of acute SCI. The behavioral test and the electrophysiological and morphological studies showed that transplantation of O-2A cells may play an important role in functional recovery

and the regeneration of axons after SCI (Lee KH, et al., 2005).The olfactory ensheathing cell is a specialized glial cell that assists in growth of the axons of the olfactory sensory neurons. There are increasing evidences in animal models that transplantation of olfactory ensheathing cell promotes recovery after transplantation into the injured spinal cord. Olfactory ensheathing cell transplantation has promoted regrowth of axons across the injury site and led to recovery of functional behaviours including climbing, walking, reaching and breathing. Most evidences of olfactory ensheathing cells derived from the olfactory bulb indicated that olfactory bulb is an impractical site for human biopsy compared to the olfactory mucosa in the nose. After devising methods to grow human olfactory ensheathing cells from nasal biopsy could in-vitro be cultured, Mackay-Sim et al recently initiated a phase I clinical trial of human olfactory ensheathing cells transplantation into the human paraplegic spinal cord (Mackay-Sim A, 2005).Scientists transplanted Olfactory ensheathing cells (OECs) prepared from the olfactory bulbs of adult transgenic SD rats into dorsal spinal cord transection lesion of SD rats. And then myelinated axons spanning the lesion were observed in discrete bundles encapsulated by a cellular element. Open-field locomotor behavior was significantly improved in the OEC transplantation group. Thus, transplanted OECs derived from the adult olfactory bulb could survive and orient longitudinally across a spinal cord transection site and form myelin. This pattern of repair is associated with improved locomotion(Sethi R, et al., 2014).

Woodhouse et al reported that the proliferation of ensheathing cells was significantly increased when cocultured with explants from uninjured spinal cord and spinal cord that had been subjected to chronic contusion or chronic needle stab injury, but not to acute needle stab injury. Apoptosis of ensheathing cells was significantly increased when cocultured with acutely stabbed spinal cord explants, which suggested that delaying transplantation after spinal cord injury may be beneficial to ensheathing cell survival (Woodhouse A, et al., 2005).Cao et al reported for the first time that the genetically modified OECs are capable of producing GDNF in-vivo to significantly improve recovery of spinal cord injury. After implantation into the spinal cord of adult rats with complete spinal cord transection (SCT), the locomotor functions of animals and axon regeneration were assessed. This work combined the outgrowth-promoting property of OECs with the neuroprotective effects of the additionally overexpressed neurotrophic factors, opening new avenues for the treatment of spinal cord injury (Cao L, et al., 2004). Barakat et al compared the effects of Schwann cells (SCs) and olfactory ensheathing glia (OEG) in facilitating the growth of supraspinal and afferent axons and promoting restitution of hind limb function after transplantation into the chronically contused spinal cord. They found that animals with SCs transplantation recovered better than OEG groups (Barakat DJ, et al., 2005).

Joosten et al used adult female Wistar rats that received a dorsal hemisection at thoracic spinal cord levels to study the function of neonatal astroglial cells. They found that

the presence of transplanted neonatal astroglial cells resulted in a significant increase in the number of ingrowing neurofilament-positive fibers into the implant. Ingrowing fibers were closely associated with the transplanted astroglial cells. The implantation of neonatal astroglial cells resulted in modest temporary improvements of locomotor recovery as observed from open-field locomotion analysis (Joosten EA, et al., 2004). Mikami et al reported a treatment of spinal cord injury involving implantation of dendritic cells (DCs). In DC-implanted adult mice, endogenous NSPCs in the injured spinal cord were activated for neuroregeneration. These DCs produced neurotrophin-3 and activated endogenous microglia in the injured spinal cord. Behavioral analysis revealed that the locomotor functions of DC-implanted mice significantly recovered as compared to those of control mice. These results suggested that DC-implantation exerted trophic effects including activation of endogenous NSPCs, leading to repair of the injured adult spinal cord (Mikami Y, et al., 2004).The data from Zhao et al first demonstrated that intraspinal transplantation of CD34+ human umbilical cord blood cells promoted the functional recovery after spinal cord hemisection in rats and these cells might be an excellent choice as routine starting material of allogenic and autologous transplantations for the treatment of spinal cord injury (Zhao ZM, et al., 2004).Cell-based approaches to solve the problem in treatment of spinal cord injury have been studied widely in the world. Although no definite decisions on which adult stem cells are most effective for this CNS injury, their ability to differentiate and improve locomotor recovery holds promise for a cure.

Virus-based approaches
Nowadays, a lot of studies focused on the method using the harmless virus-based vectors to investigate proper treatments. Poliovirus-based vectors (replicons) have been shown to maintain the in-vitro tropism of poliovirus for motor neurons of the CNS. Animals with spinal cord trauma receiving replicons encoding IL-10 showed a better functional recovery in the first 24 h after injury, which was maintained throughout the testing period. Results of these studies demonstrated that replicons could be used to express biologically active molecules in motor neurons of the CNS which had a direct effect on the CNS or inducing a cascade of molecules that can influence the cellular composition within the CNS (Price T J, et al., 2012). Neurotrophins can promote axonal regeneration, but the techniques available for delivering neurotrophins have limited effectiveness. Kodaet al studied the effect of adenovirus vector mediated gene transfer of brain-derived neurotrophic factor (BDNF) on axonal regeneration inn SCT rats at the T8 level. Axonal regeneration after transection was assessed by retrograde and anterograde tracing. The tracing experiments demonstrated that rats with significant locomotor recovery of hindlimb function occurred regeneration of rubrospinal axons, a descending axons, not just a simple enhancement of the central pattern generator (Koda M, et al., 2004) Tang et al assessed the effect of GDNF mediated by a recombinant adenovirus on the functional recovery and central neuronal

atrophy in bilateral corticospinal tracts electrolytic lesions at the T10 vertebral level in adult rats. After 2-3 weeks, the neurological score and the inclined plane angle were significantly higher and the soma size of corticospinal motoneurons was larger in the transplanted group compared to the control group. So this might be an approach to prevent the retrograde atrophy of corticospinal motoneurons and improve the motor function in rats with spinal cord injury(Tang XQ, et al., 2004). Ruitenberg et al first showed that adeno-associated viral (AAV) vector can be used for the persistent transduction of highly atrophic neurons in the red nucleus (RN) for up to 18 months after injury. Furthermore, BDNF gene transfer into the RN following spinal axotomy resulted in counteraction of atrophy in both the acute and chronic stage after injury. These novel findings demonstrated that gene therapeutic approach can be used to reverse atrophy of lesioned CNS neurons for an extended period of time(Ruitenberg MJ, et al., 2004).

Inhibitory or antibodies
It is thought that there are some inhibitory factors preventing the regrowth of axon of neurons, and some studies have been involved in neutralize the inhibitory factors and give the injured neurons much beneficial microenvironment. Members in Nogo family are a group of inhibitories in preventing regrowth of axons. Axon growth after spinal injury is thought to be limited in part by myelin-derived proteins that act via the Nogo-66 Receptor (NgR). Li et al studied the recovery from mid-thoracic dorsal over-hemisection injury after inhibiting NgR transgenically with a soluble function-blocking NgR fragment. And their results indicated that the NgR ligands, Nogo-66, MAG, and OMgp, played a role in limiting axonal growth in the injured adult CNS and that NgR might provide a therapeutic means to promote recovery from SCI (Li S, et al., 2005). Fouad et al examined the effects of an extensively used anti-Nogo-A antibody (mAb IN-1) on the regenerative capabilities of unilateral thoracic lesioned corticospinal tract axons in the Marmoset monkey. The labeled neurites in the mAb IN-1-treated animals had grown into, through and around the lesion site. This was the first anatomical evidence that in primates, the neutralization of the myelin-associated inhibitor Nogo-A resulted in increased regenerative sprouting and growth of lesioned spinal cord axons (Fouad K, et al., 2004).
After spinal cord injury (SCI), the acute inflammatory response mainly caused the damages of descending pathways. Increases in serotonergic fiber density in rostral spinal segments and decreases in caudal to the lesion have been observed previously, contributing to neuropathic pain and motor dysfunction associated with SCI. Oatway et al investigated the effect of an acute anti-inflammatory treatment on the density of serotonergic fibers in rostral and caudal to a thoracic SCI lesion. Finally, they found that the anti-CD11d integrin antibody treatment was neuroprotective after SCI, corresponding with improved patterns of intraspinal serotonergic innervation. The improvement in

serotonergic fiber projections paralleled reduced mechanical allodynia and enhanced locomotor recovery (Oatway MA, et al., 2005). Using a rat spinal cord injury model, some studies were devised an anti-inflammatory treatment to block the infiltration of neutrophils and hematogenous monocyte/macrophages on the first 2 days post-injury by targeting the CD11dCD18 integrin. This agent reduced neutrophil and macrophage infiltration effectively. Anti-CD11d mAb treatment following SCI would minimize the destructive actions associated with early, uncontrolled leukocyte infiltration into the lesion while permitting the positive wound healing effects of macrophages (Saville LR, et al., 2004).Okada et al assessed the efficacy of rat anti-mouse IL-6 receptor monoclonal antibody (MR16-1) in the treatment of acute contusive SCI in mice. The lesions were assessed histologically and the functional recovery was evaluated. Their results showed MR16-1 could inhibit the development of astrogliosis after SCI, decrease the number of invading inflammatory cells and the severity of connective tissue scar formation. And significant functional recovery in the mice treated with MR16-1 compared with control mice was observed. These findings suggested that neutralization of IL-6 signaling in the acute phase of SCI represented an attractive option for the treatment of SCI (Okada S, et al., 2004).Fibrous lesion scar plays a pivotal role as a growth barrier for regenerating axons in adult spinal cord. Klapka et al reported that they made transient suppression of collagenous scarring in the lesion zone by local application of a potent iron chelator and cyclic adenosine monophosphate in adult rats that received transection of the dorsal corticospinal tract at thoracic level 8. And the lesioned spinal cord showed a delay in fibrous scarring caused by local inhibition of collagen biosynthesis and basement membrane deposition which was a promising and unique therapeutic strategy for treating human spinal trauma (Hagg T, et al., 2005).To assess the potential effect of P2X7 purine receptors (P2X7R) blockade on ameliorating acute spinal cord injury (SCI), Wang et al delivered P2X7R antagonists OxATP or PPADS to rats after acute impact injury. The observations demonstrated that SCI was associated with prolonged purinergic receptor activation, which results in excitotoxicity-based neuronal degeneration. P2X7R antagonists could inhibit this process, reducing both the histological extent and functional sequelae after acute SCI (Wang X, et al., 2004).

Murakami et al showed the effect of the protein tyrosine kinase inhibitor lavendustin A on axonal growth of neurons differentiated from neural stem cells. Significantly greater axonal outgrowth was observed form the neurons treated with the inhibitor. Thus, protein-tyrosine kinase inhibition was effective for axonal outgrowth of neurons and might prove to be useful for neuronal regeneration via transplanted stem cells, particularly in the case of spinal cord injuries (Murakami K, et al., 2004).

Combination methods

Though either the cell-based approaches or treatment by virus-based vectors solitary, there was no satisfying results come out. Scientists tried to use the combination of two or more methods, and hope to find some more beneficial approaches. Scientists use adenoviral (AdV) vectors encoding neurotrophin-3 (AdV-NT-3) or the bacterial marker enzyme beta-galactosidase to transducer OEG cultures. Engineered cell suspensions were then injected into adult Fischer 344 rat spinal cord immediately after unilateral cervical (C4) corticospinal tract (CST) transection. And they found that OEG transplantation per se can promote tissue sparing after injury, but, after appropriate genetic modification, these olfactory-derived cells become far more effective in promoting long-distance maintenance/regeneration of lesioned adult CST axons (Barbour H R, et al., 2013)Others infected rat embryonic (14d) spinal cord-derived glial-restricted precursor cells (GRPs) with retroviruses expressing the multi-neurotrophin D15A (with both BDNF and NT3 activities). And the results showed combined treatment with neurotrophins and GRP grafts could facilitate functional recovery after traumatic SCI and prove to be a useful therapeutic strategy to repair the injured spinal cord (Yasuda A, et al., 2011).Transplantation of neural precursor cells (NPCs) into lesioned spinal cord results in only partial functional recovery, and most transplanted cells tend to differentiate predominantly into astrocytes. In order to improve functional recovery after transplantation, it is important that transplanted neural precursor cells appropriately differentiate into cell lineages required for spinal cord regeneration. In order to modulate the fate of transplanted cells, Setoguchi et al advocated transplanting gene-modified neural precursor cells. And they demonstrated that gene modification to inhibit bone morphogenetic protein (BMP) signaling by noggin expression promoted differentiation of neural precursor cells into neurons and oligodendrocytes, without astrocytes after transplantation. Their observations suggested that gene-modified neural precursor cells that express molecules involved in cell fate modulation could improve central nervous system (CNS) regeneration (Setoguchi T, et al., 2005). Zeng et al co-transplanted neural stem cells with schwann cells (SCs) into the injured spinal cord and found that SCs can promote the survival and differentiation of transplanted neural stem cells in the injured spinal cord (Zeng YS, et al., 2005).The combination therapy may overcome a central limitation of transplant strategies in which the permissive environment was provided at the implantation site. For example, Azanchi et al injected anti-galactocerebroside antibodies plus complement proteins into the dorsal column cranial and caudal to the injury site three days after contusion injury, resulting in complete and well defined regions of demyelination that extended 8 mm either side of the injury site. One day later, naive Schwann cells in suspension were injected into the contusion site. Animals that received demyelination plus transplantation therapy exhibited robust axonal regeneration beyond the contusion site within the treated dorsal column. Axonal regeneration in these animals was not associated with the improvement of locomotor ability (Azanchi R, et al., 2004). Methylprednisolone (MP) and interleukin-10 (IL-10) are tissue protective acutely after

spinal cord injury; however, the beneficial effects of MP/IL-10 were not always additive when combined with cell transplantation (Hill C E, et al., 2012).

Therapeutic electrical stimulation

During the last one-half century, electrical stimulation has become clinically significant for improving health and restoring useful function after spinal cord injury (Creasey GH, et al., 2004).Short-term stimulation can be provided by electrodes on the skin or percutaneous fine wires, but implanted systems are preferable for long-term use. Electrical stimulation of intact lower motor neurons can exercise paralyzed muscles, reverse wasting, improve strength, endurance and cardiovascular fitness, and reduce the progression of osteoporosis. Stimulation of intact sacral nerves can produce effective micturition and reduce urinary tract infection. Electroejaculation can now produce semen in most men with spinal cord injury. Significant achievements have also been made in restoring limb function. Useful hand grasp can be provided in C5 and C6 tetraplegia, reducing dependence on adapted equipment and assistants. Standing, assistance with transfers, and walking for short distances can be provided to selected persons with paraplegia, improving their access to objects, places and opportunities which are inaccessible from a wheelchair (Creasey GH, et al., 2004). Therapeutic electrical stimulation was found to have a facilitatory effect on the muscle sympathetic nerve activity in the severed rats at T12/T13 disk level, whereas regulatory function was activated by the sympathetic nerves (Mikami Y, et al., 2005). Fujiki et al explored the influence of preconditioning and subsequent electrical stimulation on the formation of primary and secondary lesions following spinal cord hemisection injury in rats. Preconditioning electrical stimulation of the spinal cord activated reactive astrocytes at 1 week after injury in the rats treated with electrical stimulation. We may conclude that preconditioning with electrical stimulation prevents the formation of secondary lesions after spinal cord injury. This beneficial effect may be related to the ability of electrical stimulation to attenuate trauma-induced cellular cascades (Fujiki M, et al., 2004). Scientists also investigated urinary bladder and urethral sphincter responses evoked by bladder distention, ventral root stimulation, or microstimulation of S2 segment of the sacral spinal cord under alpha-chloralose anesthesia in cats with an intact spinal cord and in chronic spinal cord injured cats 6-8 weeks after spinal cord transection at T9-T10 spinal segment. Electrical stimulation of the ventral roots revealed that the S2 sacral spinal cord was the most effective segment for evoking large amplitude bladder contractions or voiding in both types of cats. The effectiveness of spinal cord microstimulation with a single electrode demonstrated the potential for using microstimulation techniques to modulate lower urinary tract function in patients with neurogenic voiding dysfunctions (Tai C, et al., 2004).These reports give us some indications that therapeutic electrical stimulation might be a very useful and powerful approach to treat spinal cord injury.

Others

Tsai et al examined the biocompatibility and regenerative capacity of synthetic hydrogel tubular devices for the first time. The devices were implanted into T8-transected spinal cords of adult SD rats. Gross and microscopic examination of the spinal cords showed continuity of tissue within the synthetic guidance channels between the cord stumps at 4 and 8 weeks. Neurofilament stained axons were visualized within the bridging tissue, and serotonergic axons were found in the channel. Retrograde axonal tracing revealed regeneration of axons from reticular, vestibular and raphe brainstem motor nuclei(Tsai EC, et al., 2004) . Results showed that axons from brainstem motor nuclei regenerated in unfilled synthetic hydrogel guidance channels after complete spinal cord transection, giving us a suggestion that synthetic devices may be an possible approach the solve the problem of spinal cord injury. Lu et al investigated the effect of liposome-mediated GDNF gene transfer in-vivo on spinal cord motoneurons after spinal cord injury in adult rats. RT-PCR and fluorescence observation confirmed the presence of GDNF cDNA at 1 week and 4 weeks after injection. GDNF gene transfer in-vivo can protect motoneurons from death and degeneration induced by incomplete spinal cord injury as well as enhanced locomotion functional restoration of hind limbs. Therefore, liposome-mediated delivery of GDNF cDNA might be a practical method for treating traumatic spinal cord injury (Lu KW, et al., 2004).

Conclusion

Injuries to the adult mammalian spinal cord often lead to severe damage of both ascending (sensory) pathways and descending (motor) nerve pathways without the perspective of complete functional recovery. Although many experiments have been involved to research effective therapy after spinal cord injury, studies have not demonstrated convincingly that cell-based approaches or other pharmacological agents really have clinically significant and important benefits for patients suffering from SCI. Future spinal cord repair strategies should comprise a multi-factorial approach addressing several issues, including optimalization of survival and function of spared central nervous system neurons in partial lesions and the modulation of trophic and inhibitory influences to promote and guide axonal regrowth (Hendriks WT, et al., 2004).

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