

An update on spinal cord injury research

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Spinal cord injury (SCI) can have a range of debilitating effects and permanently alter the capabilities and quality of life of survivors. The first specialized centers of care for SCI were established in 1944 and since then an increasing amount of research has been carried out in this area. Despite this, the present treatment and care levels for SCI are not comparable to those in other areas of medicine. In the clinic, the aim of SCI treatment is primarily to limit secondary damage by reducing compression in trauma spots and stabilizing the spinal column. Currently, no effective strategy for functional recovery is offered. In this review, we focus on research progress on the molecular mechanisms underlying SCI, and assess the treatment outcomes of SCI in animal models, i.e., neurotrophins and stem cells are discussed as pre-clinical therapies in animal models. We also assess the resources available and national research projects carried out on SCI in China in recent years, as well as making recommendations for the future allocation of funds in this area.

Keywords: spinal cord injury; neurotrophins; stem cell-based transplantation; research funding

Introduction

Data from the National Spinal Cord Injury Statistical Center show that in the USA an estimated 270 000 people are living with spinal cord injury (SCI) and ~12 000 new injuries are reported annually^[1]. It is reported that China has had a ten-fold increase in SCIs caused by car crashes, construction and mining accidents in the past decade. About 60 000 new injuries are reported annually^[2]. In addition to the debilitating effects and permanently reduced quality of life, an enormous social, financial and emotional cost for the victims, their relations and the government is brought about by SCI^[3]. Despite this immense cost to society, today's clinical treatments offer only modest benefits. This is most likely due to the complex mechanisms of SCI, the relative inability of the human body to repair or regenerate neurons in the spinal cord, and the lack of adequate government funding in this area. In this review, we focus on current progress in the molecular mechanisms of SCI, and look at the treatment

outcomes of SCI in animal models.

Pathophysiological Mechanisms Underlying SCI

Normally, the primary injury of the spinal cord is due to either contusion or compression^[4]. Although the primary cause of injury can result in the death of neurons, most is due the body's response to trauma, known as secondary injury. The mechanisms underlying this have been reported and catalogued into 25 different mechanisms^[3]. The most significant of these are vascular disturbances, inflammation, lipid peroxidation, excitotoxicity, apoptosis, demyelination and ionic disturbances.

Vascular Disturbances

Vascular disturbances play important roles in secondary injury of the spinal cord, such as hemorrhage, ischemia and reperfusion-induced damage, microcirculatory disturbances, and systemic hypotension. The traumatized cord shows severe hemorrhaging predominantly in the central

grey matter, which then expands to peripheral regions, leading to necrosis^[5]. SCI also leads to acute ischemia, which may contribute to secondary degeneration^[6]. It has been reported that a major reduction in blood flow at the lesion occurs immediately after SCI^[7-9]. Furthermore, ischemia becomes progressively worse over the first few hours, and has a linear dose-response association with the severity of the injury^[3,8]. Reperfusion damage is the paradoxical damage caused by the increased levels of free radicals and other toxic byproducts during the reperfusion of blood after a period of ischemia^[10-12]. Large arteries are unaffected, but a major change in the local microcirculation from the disruption of small blood vessels and hemorrhage leads to a failure of autoregulation and glutamate-mediated excitotoxicity^[3,13]. In addition, severe systemic hypotension can aggravate the dysfunction of microcirculation and exacerbate injury.

Inflammation

Any lesion in the central nervous system (CNS) can lead to failure of the blood-brain barrier, resulting in increased permeability and consequently triggering an inflammatory response^[14]. Thus, the immune system is involved in mediating CNS injury by inflammation through the cumulative effects of immune cells and regulatory proteins. SCIs generally induce inflammation, which is deleterious to tissue recovery and aggravates the progressive necrosis of cells in the damaged area. The extent of inflammation is increased by pro-inflammatory cytokines secreted by immune cells, including interleukin (IL)-1 β , IL-6 and tumor necrosis factor- α , which are all capable of leading towards apoptotic cell death^[3].

Four major categories of immune cells are recruited by the inflammatory response: neutrophils, monocytes, microglia and T-lymphocytes^[15,16]. Neutrophils are the first to arrive at the site of injury through the circulatory system. In addition to removing microbial intruders and tissue debris, they release cytokines, proteases and free radicals that induce further inflammation and involve glial cells in the inflammatory cascade, which ultimately leads to neuronal injury or death^[3,17]. Then monocytes infiltrate into the spinal cord and differentiate into macrophages, and, together with activated resident microglia (differentiated macrophages in the CNS), also secrete cytokines, free radicals and growth factors. The role of T-lymphocytes in SCI is rather contro-

versial^[3,15,18,19]. Following SCI, activated microglia become efficient antigen-presenting cells, which bind to T-cells when they pass through the blood-brain barrier. T-cells also release neurotrophins and modulate microglia or macrophages which function to protect neurons from degeneration^[14,20,21].

Glial-Associated Damage

Demyelination following SCI aggravates the damage in a traumatized cord since the loss of myelin exposes axons to the injurious surroundings, leading to neuronal loss via necrosis and/or apoptosis^[3]. It also delays or even blocks the conduction along axons resulting in inefficient communication between neurons^[22,23]. Demyelination is due to the loss of oligodendrocytes, whose death is triggered by glutamate excitotoxicity and exacerbated by the inflammatory cascade. Hence, further understanding of demyelination and oligodendrocyte-caused neuronal loss is fundamental to improving the treatment and cure for SCI^[3,22].

Inflammatory reactions subside eventually and glial scar tissue forms. Hours after SCI, astrocytes in the lesion site proliferate and form glial scars, which can isolate neural tissue from inflammatory cells and decrease neuroinflammation during the early phases^[24]. However, this scar tissue is another obstacle for neurite outgrowth which continuously develops for several months after SCI. Moreover, the inhibitory molecules secreted by these scar cells prevent functional recovery^[24]. Actually, there are many sorts of molecules, covering the scar and its surroundings, that prevent injured axons from regenerating, and the net effect of degeneration is likely to be amplified in the traumatized spinal cord^[3].

Necrosis and Apoptosis

Apoptosis is a natural physiological process that plays a key role in the developmental maintenance of cells and the spinal cord. In the primary phase of SCI, many types of cells undergo necrosis and apoptosis, while in the secondary phase, apoptosis is mainly limited to the white matter. The apoptotic cascade in SCI is activated in neurons, oligodendrocytes, microglia, and, perhaps astrocytes^[25,26]. A major trigger appears to be the injury-induced calcium influx, the loss of ionic homeostasis, and the increased excitotoxicity following SCI, which all trigger apoptotic cell death and mitochondrial dysfunction^[27-30]. Recent studies have reported that apoptosis in SCI is primarily mediated by Fas, thus its inhibition may be a potential therapeutic strategy^[31,32].

Others

Lipid Peroxidation and Oxidative Stress

The level of free radicals, which are one of the main mediators of axonal disruption in SCI, increases in the lesion site. As spinal cord tissues are rich in lipids and sensitive to lipid peroxidation, free radicals can cause cell membrane lysis by absorbing an electron from a lipid molecule to make it less stable^[3]. In physiological states, healthy tissues generate some free radicals, which are effectively removed by endogenous oxidative systems, while in SCI, excessive free radicals are produced because of the derangements in energy metabolism^[33]. Hence, too many free radicals in SCI ultimately lead to the lysis of cell membranes and necrosis through lipid peroxidation (oxidative stress). In addition, oxidative damage aggravates mitochondrial dysfunction and causes intracellular calcium overload^[3,30,34].

Calcium-Associated Disorder

Calcium influx is involved in excitotoxicity and ion imbalance, both of which are main mechanisms of SCI. It is triggered by acute injury and continues for hours to weeks afterwards^[17].

Glutamate, the major excitatory neurotransmitter in the CNS, is released excessively after SCI. The abnormally high level of extracellular glutamate causes direct damage to the spinal cord and increases calcium influx. This leads to neuronal death by necrosis or apoptosis through the process known as excitotoxic cell death (excessive glutamate stimulates the NMDA and AMPA receptors in the postsynaptic membrane)^[13,35]. Neurons and oligodendrocytes are particularly vulnerable to glutamate excitotoxicity since they express a full complement of glutamate receptors^[3]. As a result, excitotoxic injury brings about the demyelination of axons and loss of neurons. Furthermore, nitrous oxide produced by iNOS after SCI is also involved in glutamate excitotoxic injury^[36].

The derangements of ionic homeostasis in SCI that are detrimental to cell function and survival include increased calcium influx, potassium outflow and intercellular accumulation of sodium^[37]. An excessive intracellular level of calcium ions is a key element in the secondary injury mechanism. It is considered that calcium influx is the final shared pathway that leads to cell death. Hence, the inhibition of calcium influx is a viable therapeutic strategy for SCI.

All the above, primary and secondary damage con-

tribute to the dysfunction of spinal cord injury, so improved understanding of the complex mechanisms underlying both are imperative for the development of clinical methods.

Research Progress in the Treatment of SCI

Neurotrophins

Neurotrophic factors are regulators of neuronal plasticity and regeneration, and their expressions are modified after SCI^[38]. Therefore, they have been used to both promote axonal growth and prevent neuronal death. To date, researchers have carried out experiments on the classic neurotrophin family that includes typical neurotrophic factors, like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3)^[39]. They have also been applied in cell transplantation to promote tissue regeneration after SCI.

Nerve Growth Factor

NGF promotes the sprouting and regeneration of axons^[40–42]. And exogenous delivery of NGF to animal models of SCI can promote robust axonal growth^[40]. NGF moderately promotes neuronal survival in certain nuclei^[43]. It also has stimulatory effects on the sensory fibers in the spinal white matter after SCI^[44].

Both methylprednisolone and erythropoietin are currently used in SCI treatment. Erythropoietin partially contributes to improved recovery of motor functions *via* the increased expression of NGF. However, methylprednisolone and saline do not have such effects^[45]. In contrast, anti-NGF treatment prevents the development of abnormal somatosensory behavior, which suggests a potential preemptive analgesic treatment for central pain^[46].

Furthermore, neutralizing NGF in the cord can minimize the life-threatening autonomic dysreflexia following SCI^[47]. Therefore, using NGF for therapy should take all possibilities into account.

Brain-Derived Neurotrophic Factor

BDNF can reduce the necrotic zone and help neuronal survival after SCI^[48]. Like NGF, exogenous delivery of BDNF to animal models of SCI can promote robust axonal growth^[49]. BDNF has both short- and long-term effects on neuronal regeneration and functional recovery^[50]. For instance, local application of BDNF decreases loss of function in the partially-transected rat spinal cord starting one day after SCI^[50]. And BDNF, playing a neuroprotective role, is synthe-

sized in both neurons and astrocytes during the acute response to SCI^[51]. Moreover, even delayed BDNF treatment has an anti-apoptotic effect in oligodendrocytes after SCI, which suggests that it is capable of suppressing secondary injury^[52].

BDNF-GFP transgenic stem cells have better outcomes than stem cells alone for SCI^[52]. Bone marrow stromal cell grafts secreting BDNF also promote the regeneration of some neuronal populations^[38].

Furthermore, continuous intramedullary infusion of BDNF provides neuroprotection and enhances some regenerative activity after SCI^[54]. And continuous infusion of BDNF after initial methylprednisolone treatment improves functional recovery after severe SCI without reducing the therapeutic effect of methylprednisolone^[55].

Neurotrophin-3

NT-3, like BDNF, promotes the regeneration and overexpression of NT-3 in the rat spinal cord, which subsequently induces sprouting of corticospinal tract axons^[56]. Furthermore, the delivery of a single dose of NT-3 at the time of spinal cord lesion induces sprouting of corticospinal axons^[57].

Much more research has been undertaken on the use of NT-3 in SCI therapy. NT-3 plays a protective role in the injured CNS through interaction with trk receptors. The multi-neurotrophins NT3/D15A have the capacity to bind both trkB and trkC and have positive effects on cell survival and remyelination after SCI. This is indicative of a possible treatment therapy in the future if it is combined with cell transplantation^[58].

Stem Cell Transplantation

Due to the cavitation resulting from SCI, a better treatment strategy can be filling the physical gap by replacing its lost elements and supporting tissues with various cell transplants^[59]. This can contribute to the remyelination of axons, including embryonic stem cells (ESCs), adult neural precursor cells (NPCs), induced pluripotent stem cells (iPSCs), and oligodendrocyte precursor cells (OPCs), as well as Schwann cells, olfactory ensheathing cells, and bone marrow stromal cells^[60,61].

Human Embryonic Stem Cells

ESCs, with self-renewal potential and genetic adaptability, have the capacity to differentiate into nearly all types of cells, including motoneurons and glial fate cells, which thus make them an attractive source for treating neurological

disorders and trauma such as SCI^[14,62-64]. The demyelination and loss of myelinated cells cause abnormal neuronal function, since oligodendrocytes are highly vulnerable to the factors existing in the sites of trauma and may undergo apoptosis or necrosis^[14].

In the late 1990s, the successful isolation and differentiation of human ESCs showed much promise for regenerative therapy for CNS injuries and created much interest in this area. Since then, this technique has been shown to be effective in restoring function in various animal models^[65]. Human ESCs transplanted into Parkinsonian rats become integrated functioning cells in the nervous system^[66]. In SCI, human ESCs are one of the most attractive strategies for promoting cell survival and integration into the spinal cord^[42]. It seems that their beneficial effects are not due to remyelinating activity, but through fostering a neuroprotective environment^[67]. Human ESC-derived oligodendrocyte transplantation activates the BDNF and IL-6 signaling pathways^[68].

An outstanding advantage of human ESCs is that they are able to generate NPCs, including regionally specific neurons, guaranteeing that human ESC-derived NPCs are more effective in transplantation than adult neural stem cells (NSCs) following SCI^[13]. However, challenges remain that should be taken into consideration before they can be applied in the clinic. These include the potential of human ESCs to cause tumorigenesis, ethical issues and rejection by the host immune system^[69].

Neural Stem Cells, Neural Precursor Cells, Neuronal Restricted Progenitor Cells, Oligodendrocyte Precursor Cells and Glial-Restricted Progenitor Cells

NSCs can be efficiently propagated *in vitro* and have the potential to differentiate into neurons, oligodendrocytes and astrocytes^[70,71].

NPCs can be differentiated into both neuronal and non-neuronal lines, while NRPs, which are more differentiated NPCs, can only be differentiated into neurons^[59]. When NPCs are transplanted into the permissive neuronal environment of the dentate gyrus or subventricular zone, they differentiate into neurons. When they are transplanted into a non-permissive environment, like that of an SCI, they usually differentiate into a glial line^[72-74].

Hence, in therapy for SCI, endogenous or transplanted

NSCs differentiate mostly into oligodendrocytes and astrocytes. The majority of transplanted NPCs differentiate along the oligodendroglial lineage^[75]. The dentate gyrus is a source of this cell type^[76]. Transplantation of glial-restricted progenitor (GRP) cells and OPCs differentiated from NSCs, results in remyelination and functional repair following SCI^[77].

In rodent SCI models, NPC-derived oligodendrocytes ensheath injured axons, generate new myelin, and promote locomotor recovery. These cells can also myelinate the congenitally-dysmyelinated spinal cord of the shiverer mouse^[69,78]. However, there are still two important barriers to endogenous OPC remyelination. One is the glial scar that blocks the access of OPCs to demyelinated axons; the other is the expression of inhibitory molecules by astrocytes, which inhibit OPC differentiation and proliferation^[14,79].

Compared to oligodendrocytes, astrocytes, which are also differentiated from GRP cells, play a role in the mediation of neuroprotection. They support axonal regeneration and decrease focal motor neuron loss by secreting many neurotrophic factors^[80,81].

Recruitment of endogenous NSCs or transplantation of NSCs is another strategy for the treatment of SCI. In fact, NSCs maintain their capacity for stable self-renewal after several passages *in vitro*, so it seems they have less potential for tumor formation than ESCs^[81]. There are still, however, many critical challenges that have to be faced: (1) the decreased potential of differentiation after several passages, (2) the need for pure neuronal populations of differentiated cells, (3) the formation of glial scars, (4) the inhibitory molecules secreted by astrocytes, (5) inefficient attacking systems, and (6) moderate cell survival after transplantation^[14,71,82].

Induced Pluripotent Stem Cells

Current cell-based approaches have to overcome a number of critical problems before clinical application, including ethical concerns, tumor formation and immunological rejection.

iPSCs are modified cells used for transplantation and have the capacity to differentiate into all kinds of cells like ESCs, e.g. neurons, glia, NPCs, and motoneurons^[83,84]. Initially, they were generated to overexpress defined factors (Oct4, Sox2, Klf2, and c-Myc) using retroviral transfection^[85]. These factors are sufficient to reprogram somatic

cells to a pluripotent state. iPSCs have been obtained from different sources, e.g. mice, rats, monkeys, and humans^[86-91]. When they are derived from patient-specific adult somatic cells, there is no danger of host rejection in autologous transplants, and they circumvent ethical problems that are often associated with obtaining ESCs by destroying an embryo. However, there are two big barriers to the clinical application of iPSCs. One is the introduction of transgenes^[92]. It has also been noted that their global gene expression and histone methylation differ from their ESC controls. It is known that epigenetic modifications of methylation and de-methylation act to open and close DNA to gene transcription. The other is teratoma formation. iPSCs may have even greater tumor potential than ESCs, as a result of genetic alteration^[69,93].

Consequently, iPSCs can be seen as superior to ESCs in many ways. In fact, pre-clinical studies of the safety and effectiveness in *in vitro* models and *in vivo* are not sufficient. *In vivo* application of human iPSCs is the ultimate goal of regenerative medicine in SCI, and there is no reason why this could not be achieved in the near future with adequate funding and resources.

An Analysis of Funding for SCI Projects in China

In order to assess the funding situation in China, we analyzed data from the central database of the National Natural Science Foundation of China (NSFC). We thus found that national funding for SCI projects is increasing, but the increase is not in line with other areas of medical research.

The NSFC was founded in 1986, but at this time SCI was not an area of medicine receiving much emphasis. Since then, a total of 297 projects (Fig. 1A) have been carried out in this field and 74 525 000 RMB (US\$11 904 952) (Fig. 1B) spent on it. In recent years, ~50 projects/year have been carried out. The distribution of these projects is by no means uniform across China. They are primarily done in high-level universities and research institutions. The proportion of government funds spent on this area is significantly lower than that in western countries. Furthermore, the absence of activities against research in this area on religious grounds makes China an ideal country to make groundbreaking discoveries and develop pioneering therapies for victims of SCI.

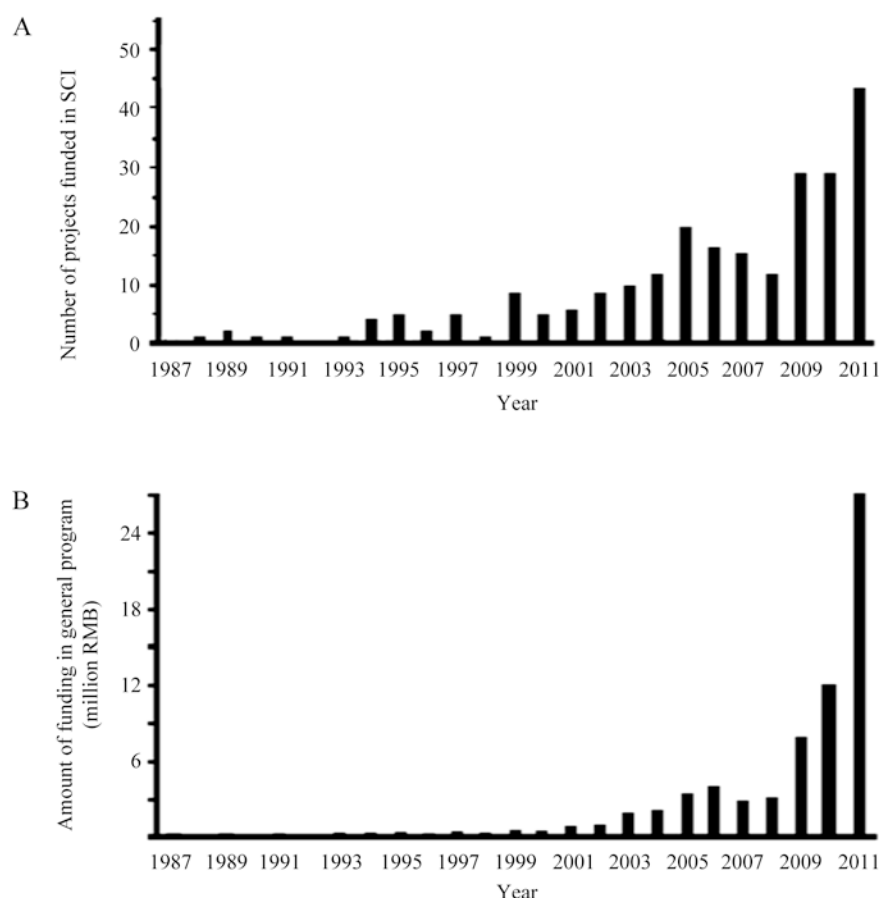


Fig. 1. The distribution of the funded projects in recent decades and the trend of NSFC funds growth in the field of spinal cord injury (SCI). A: The number of projects funded in SCI; B: Amount of funding in general program (million RMB).

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