

Novel strategies for protection and repair of the central nervous system

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ABSTRACT – Clinical possibilities in many neurological conditions are limited by our current inability to correct structural damage to the nervous system, and treatments to prevent damage are also limited. Current research has produced promising treatments that promote neuroprotection, plasticity, axon regeneration, remyelination and cell replacement. As these treatments go through clinical trials and enter the clinic, the treatment of several neurological conditions will change greatly.

KEY WORDS: Alzheimer’s disease, axon regeneration, head injury, multiple sclerosis, plasticity, spinal cord injury, stem cells, stroke

Introduction

The large number of conditions that can damage the central nervous system (CNS) range from those affecting the fetus through neurodegenerative disease to traumatic damage. The damage caused by these conditions can be divided into three main categories:

- damage to neurons
- damage to connections
- damage to myelin.

How to prevent or repair these forms of damage has been an extremely active field of research for many years and great advances have been made in developing scientific knowledge. However, in only a few instances have these advances so far led to treatments that benefit patients. This will change rapidly in the next decade, because many of the scientific advances are now leading to novel treatments that will enter clinical trials and then reach the clinic.

Protection

Stroke, head injury, spinal cord injury and the other acute forms of damage to the CNS create a central lesion area which cannot be protected. Following this initial event there is a much longer-lasting process which considerably expands the lesion, doubling its size in some instances. In this expanding area, known as the penumbra in stroke lesions, there is a process of progressive cell death which may continue for a week

or more. Much of the effort in CNS protection has been directed towards preventing this progression. Four types of intervention have been attempted.

- 1 Excitotoxicity is caused by the uncontrolled release of glutamate from neurons and glial cells which occurs in ischaemic and damaged regions;¹ the continued stimulation of neurons places an unsupportable metabolic load on neurons and leads to the entry of dangerous quantities of calcium. Several compounds blocking the NMDA receptor, a glutamate-activated channel which admits calcium, were effective in animal models of stroke, but failed in clinical trials. In retrospect, it is not clear whether these failures were because the compounds were ineffective or the trials unable to detect their effects. Other forms of glutamate antagonist and compounds designed to combat excitotoxicity have been developed, but few have reached clinical trials.
- 2 When cells are challenged by toxic events they may initiate a cell death programme, the best understood of which is apoptosis. This process leads to the release of cytochrome C from mitochondria, triggering cell suicide.² Many interventions in this pathway – from the application of growth factors that oppose apoptosis, to treatments to prevent release of cytochrome C, to molecules blocking the action of the caspases which execute the apoptosis pathway – have been applied to stroke and other disease models. Many have had effects in reducing the size of infarcts in animal models, but the treatments have not yet reached clinical trials.
- 3 A third form of toxicity is the release of reactive oxygen species or free radicals.³ Again, compounds have been developed which intervene in this process one of which, NXY-059, a free radical trapping compound, has shown protection in a phase 2 trial and is currently undergoing phase 3 trials (www.renovis.com).
- 4 Finally, the method that is used in clinical practice to ameliorate brain damage after head injury and stroke is the optimisation of

physiology. The first priority is to remove thromboses that are preventing blood flow, for which thrombolytics are routinely used. Around a stroke or area of trauma there is a region of disturbed blood flow, and imaging of oxygen utilisation shows that these regions are often ischaemic. Prevention of raised intracranial pressure, and maintaining a high pCO₂ level to benefit from its vasodilator effects, are among the interventions that have been helpful.⁴ A list of current clinical trials in stroke is available at www.strokecenter.org/trials.

The other main source of damage to the CNS is chronic neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and motor neuron disease. The treatment of these conditions is outside the scope of this article, but there is intense activity in the development of protective compounds for Alzheimer's disease, focused on modifying the activity of the secretase enzymes that cleave Alzheimer precursor protein to produce the toxic Abeta peptide, and on developing immunisation treatments that clear Abeta from the brain.

Plasticity

The damaged nervous system can compensate to some extent by rewiring its connections to bypass the missing structures, a process known as plasticity. After stroke, for instance, scans for functions that used to be performed by the damaged region often show that this function has moved to the perilesional area or has become widespread.⁵ The partial return of function that occurs after stroke or head injury is largely due to this plasticity. The ability of young children to recover motor skills better than adults after damage to their nervous system is considered to be due to the greater plasticity of the infant CNS.

Until recently there were no pharmacological treatments to enhance plasticity. However, driving plasticity by intense physiotherapy is now regularly used to improve arm and hand function after stroke, for instance in constraint-induced movement therapy.⁶ This involves strapping up the good arm to force patients to use their paretic limb, and has been shown to improve the eventual function of stroke-affected upper limbs.

Also, some promising compounds to enhance plasticity have been developed recently. One treatment uses an enzyme called chondroitinase to digest inhibitory proteoglycan molecules in the extracellular matrix that surrounds neurons. Late in development, at the time that the critical periods for plasticity terminate, many neurons in the brain and spinal cord acquire a dense extracellular matrix coat, known as the perineuronal net. The way in which these structures turn off plasticity is not fully understood, but they probably act by forming a barrier that prevents new connections from being formed on the neurons, and by concentrating molecules that affect the motility of the dendritic spines that are the site of excitatory synapses.⁷ Chondroitinase injections into the spinal cord have produced considerable improvements in behavioural function in animals with partial spinal cord injuries.

A second treatment that promotes plasticity is an antibody that blocks inhibitory Nogo molecules which are found mainly

on oligodendrocytes. The way in which Nogo might block plasticity is not understood, but treatment with the antibody has produced sprouting of new connections and circuits in the injured spinal cord with positive effects on behaviour. Anti Nogo has also been used to treat stroke models in rodents, again with positive effects on function.⁸ The antibody has just entered clinical trials for spinal cord injury (www.emsci.org). An antibody to another myelin protein, myelin-associated glycoprotein (MAG), has recently been shown to be beneficial in stroke models.

A third treatment that promotes plasticity involves inosine, a small molecule related to adenosine. The mechanism of its action is under investigation, but it has been shown to stimulate sprouting of nerve fibres in the brain and spinal cord in animals with spinal cord injury and stroke.⁹ The fourth type of treatment with effects on plasticity utilises trophic factors.

All of these treatments have produced improved function in animals with damage to their brain or spinal cord, but it is not known whether they will have an additive effect if given together.

How will these treatments be used to treat patients with stroke, head injury, spinal cord injury and other conditions? At present all of them have to be delivered through an indwelling cannula directly to the CNS, which is acceptable in spinal cord injury where most patients have surgical interventions, but difficult in a common condition such as stroke where early mobilisation is important. It is not clear at present where the compounds will have their greatest effect; for plasticity in the corticospinal tract the spinal cord may be the best site of administration, while for other pathways the cortex or subcortical structures may be most effective. There are indications that it may be important to restrict the site of action of treatments. Young children have a higher level of plasticity than adults, allowing them to make remarkable motor recovery after CNS damage. However, the drawback is that reorganisation of the nervous system may be so radical that cognitive performance is affected.¹⁰ The implication is that it will be helpful to promote plasticity in motor structures, but treatment of regions involved in cognition could have negative effects. Animal experiments indicate that plasticity has to be driven by appropriate behaviours coupled with strong motivation.¹¹ Plasticity treatments will probably therefore be most effective when coupled with intensive physiotherapy aimed at improving affected behaviours. It is likely that the current treatments will be superseded by small molecule pharmaceuticals that will be easier to deliver than the current molecules, but this may take several years and the current treatments will probably undergo clinical trials before then.

Axon regeneration

Damage to axons is a common feature of CNS conditions, and the main cause of functional loss in spinal cord injury. Head injury leads to widespread axon damage, shown histologically by the presence of numerous axonal swellings full of materials that are usually transported down the cytoskeleton.¹² Stroke may also damage large numbers of axons. Some of these axons may

recover, but many are irretrievably damaged. Axon damage is also the first form of pathology in some neurodegenerative conditions. There are no specific treatments at present for preserving damaged axons, so the scientific effort has been directed towards stimulating axon regeneration. When an axon is damaged the part distal to the damage dies rapidly, but the part attached to the cell body usually survives. The need is therefore to find ways of making this axon stump produce a new growth cone, elongate, and then reconnect to an appropriate target neuron. Fortunately, although most axon growth occurs only during embryonic development, axons are generally able to regenerate to some extent after damage provided that they are surrounded by an environment that is optimal for growth. Unfortunately the environment of the damaged CNS is very inhospitable for axon regeneration, particularly in the scar tissue that forms around sites of CNS injury.¹³ Research to improve axon regeneration is focused in two main areas: removing inhibitory factors in the CNS environment, and increasing the regenerative vigour of axons.

Removing inhibitory factors

The inhibitory factors in the CNS environment come from two main sources:

- the glial scar
- the surface of oligodendrocytes.

Scar tissue contains several cell types, including astrocytes, oligodendrocytes and their precursors, microglia and meningeal cells. Each of these cells produces inhibitory molecules, the three main types being chondroitin sulphate proteoglycans (CSPGs), semaphorins and ephrins. CSPGs are molecules that consist of a protein core to which are attached sulphated sugar chains, known as glycosaminoglycans. These sugar chains are responsible for much of the inhibitory activity of the CSPGs, probably through several mechanisms. The CSPGs are barrier-forming molecules that are found in many situations, including setting up barriers to bacterial infection after injury. In response to this bacteria have evolved chondroitinases which digest the CSPG sugar chains. Chondroitinase has been used in many situations

in the damaged CNS, particularly in the spinal cord, to promote axon regeneration.¹³ Because chondroitinase promotes both axon regeneration and plasticity, it is a frontline candidate for use in CNS repair.

The other source of inhibitory molecules is the surface of the oligodendrocytes. These are found throughout the CNS, but in regions of damage the myelin debris may be particularly inhibitory. NogoA, MAG, OMgp and ephrin B3 are the major inhibitory molecules. Of these, the main focus has been on NogoA. The first clear demonstration of axon regeneration in the damaged spinal cord came from the Schwab laboratory in Zurich in 1990, when an antibody to NogoA was applied to rat cord injuries. Since that time many experiments have shown that anti-NogoA treatment promotes axon regeneration, plasticity and functional recovery after spinal cord injury.⁸ Various new antibodies have been made, and the most effective of these has recently shown efficacy in macaque monkey partial spinal cord injuries. A phase 1 clinical trial of anti NogoA is currently under way for acute spinal cord injury. The antibody is delivered for around two weeks through an indwelling cannula whose tip is at the spinal injury site.

An alternative to blocking inhibitory molecules is to replace the CNS environment with one that is more permissive to regeneration. Most CNS axons will regenerate in the presence of Schwann cells or the similar olfactory ensheathing glia. There have been many experiments in which these cells have been implanted into spinal cord injuries and both cell types have produced useful amounts of repair in some experiments.^{14,15} Some form of cell implant will be necessary to act as a bridge to allow axons to grow across spinal cord injuries in human patients, and it is likely that these cell types will form part of these bridges.

Increasing regenerative vigour

The other major focus of axon regeneration research is to increase the regeneration response of the axon. Currently the most effective treatments use trophic factors such as BDNF and NT-3, and compounds that act through the signalling pathways that control axon growth.¹⁶ Many of the inhibitory molecules

Table 1. New treatments under development for spinal cord injury.

Agent	Effects
Anti NogoA	Blocks inhibitory NogoA
Chondroitinase	Removes inhibitory sugar chains from CSPGs
C3 Rho ribosylating enzyme	Inactivates the rho GTPase which signals inhibitory effects
Neurotrophin 3	Stimulates axon growth, given by transplants of transduced fibroblasts
Inosine	Stimulates axon growth
Olfactory ensheathing glia	Provides a bridge for axon regeneration. Clinical trial in root avulsion planned
Schwann cells	Provides a bridge for axon regeneration. Effective with compounds that increase cAMP levels

CSPG = chondroitin sulphate proteoglycans; GTPase = guanosine triphosphate.

signal to the inside of the axon growth cone via a signalling pathway that goes through the small GTPase Rho. Inactivating this pathway in various places has produced axon regeneration in rodent models.¹⁷ A Rho-inactivating molecule is currently in phase 1 clinical trials, applied as a gel to the site of spinal cord injury.

Remyelination

Demyelination is the major feature of multiple sclerosis, and there is also extensive demyelination following CNS trauma and stroke. In multiple sclerosis the two aims have been to prevent auto-immune demyelination and to replace myelin after it is lost. There are many compounds in clinical use or in trials that modulate the immune system, such as beta interferon and mitoxantrone; antibodies to IL-12 and CD40 are also in trials. Clinical trials are under way with two antibodies that block the inflammatory process, and which can almost stop the occurrence of new lesion formation. These are natalizumab (Tysabri) (www.biogenidec.com) which blocks alpha4 integrin, used by inflammatory cells to exit the circulation into regions of inflammation, and alemtuzumab (Campath-1H) (www.genzyme.com) which is an anti T cell antibody.¹⁸⁻²⁰ Both antibodies are very promising for the prevention of new lesions, but both have caused some side effects which may place restrictions on their use. A full list of current clinical trials in multiple sclerosis can be found on www.nationalmssociety.org/Clinical%20Trials.asp

The CNS contains a large number of oligodendrocyte precursors, spread throughout the brain and spinal cord. These cells become activated after demyelination, divide and can then remyelinate axons. Brains from patients with multiple sclerosis generally have many plaques within them, some of which are

shadow plaques that have remyelinated, some of which have failed to remyelinate. It is not clear why remyelination fails in some lesions and not others,²¹ although the overall ability of the brain to remyelinate declines with age. The CNS does not appear to completely run out of oligodendrocyte precursors after repeated episodes of demyelination, since most plaques contain actively dividing precursors and there must therefore be other factors that prevent remyelination. Inflammation is one of the most powerful influences on remyelination, and in animal models it can restart myelination in places where it has stopped.²² However, inflammation can also lead to further demyelination.

Various transplantation strategies have been proposed for multiple sclerosis, but all are complicated by the fact that lesions are spread all over the CNS, and it is impossible to transplant cells into all the lesions. Recently, intravenous cell therapies with stem cell populations, particularly multipotential precursors and bone marrow haematopoietic stem cells, have been investigated for cell replacement, based on the finding that these cells migrate out of the circulation into inflamed tissues. This treatment is of benefit in models of inflammatory demyelination, although it appears that the effect is not primarily through remyelination but possibly through an effect of perivascular accumulations of the cells on the inflammatory process.²³

Remyelination therapy is also proposed in spinal cord injury, where there can be large regions of demyelination surrounding partial lesions. A method has been developed for deriving oligodendrocyte precursors from embryonic stem cells,²⁴ and a clinical trial of implantation of these cells is proposed (www.geron.com).

While the primary event in multiple sclerosis is demyelination, much of the disability is due to the degeneration of axons that have been stripped of their myelin. A major aim in research is to develop treatments to preserve demyelinated axons.²⁵ Probably the most effective way to do this is to remyelinate them promptly, but other supportive treatments are also being investigated.

Cell replacement

CNS pathology usually leads to the death of neurons and glial cells. In some conditions such as Alzheimer’s disease the cell loss is diffuse, while in other conditions such as stroke it is focal. The first efforts to replace lost cells have been in Parkinson’s disease, where there is loss of a small and localised population of dopaminergic neurons. Animal experiments indicated that transplantation of embryonic substantia nigra cells into animals with Parkinsonism was a very effective treatment. The cells were

Table 2. New treatments under development for stroke.

Agent	Effects
NXY-059	Free radical scavenger. Neuroprotective
Anti NogoA	Promotes plasticity
Inosine	Promotes plasticity
Anti MAG	?Promotes plasticity
DP-b99	Membrane associated calcium and zinc chelator. Neuroprotective
YM872	AMPA receptor antagonist. Neuroprotective

MAG = myelin-associated glycoprotein.

Table 3. New treatments under development for head injury.

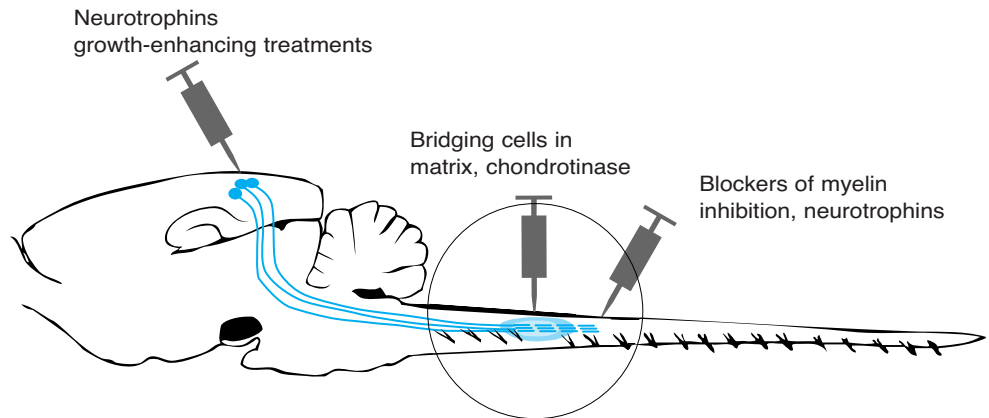
Agent	Effects
Anti MAG	?Promotes plasticity
Hypothermia	Neuroprotective
Erythropoetin	Neuroprotective

MAG = myelin-associated glycoprotein.

Table 4. New treatments under development for multiple sclerosis.

Agent	Effects
Bone marrow stem cells	Immunomodulation
Tysabri	Anti-inflammatory
Campath 1-H	Anti T cells

Fig 1. Repair of injuries to the CNS such as spinal cord injuries will eventually use multiple treatment strategies. This diagram shows a set of treatments designed to promote axon regeneration after spinal cord injury: putting together a bridge across the injury, plus treatments to remove inhibitory molecules and to stimulate the axon to regenerate.



not effective when placed in the substantia nigra, because they were unable to grow axons through the adult CNS to their target region in the striatum. However, cells placed directly in the striatum grew axons into the host brain, made connections and corrected many of the signs of the condition.²⁶ There have now been a number of trials of transplantation of human embryonic substantia nigra into the caudate putamen of human Parkinson's disease patients and several hundred patients have been treated. The results have been somewhat variable depending on the precise method used. In no case has the disease been completely cured, but in the most successful series patients have gained considerable benefit. In other series patients have suffered dyskinesias and less benefit.^{27,28} Research on improvements in technique has produced improved methods, and further transplant trials are planned.

There has been considerable interest in the possibility of using stem cells to replace lost neurons and glia. The mammalian CNS contains several endogenous stem cell populations. Around the ventricle there are multipotential stem cells whose main function is to migrate to the olfactory bulb to differentiate into neurons; in the hippocampus there are precursors that divide to continuously produce tens of thousands of new hippocampal neurons each day; and throughout the brain are the so-called oligodendrocyte precursors that can produce neurons as well as glia in some situations. While production of new neurons occurs continuously in the olfactory bulb and hippocampus, cells lost in other parts of the CNS are mostly not replaced. The adult rodent cortex has a very limited ability to replace a few neurons that are killed in a way that does not create an injury response, but this is not of sufficient magnitude to be clinically useful.²⁹ There is considerable interest in finding ways to manipulate the endogenous stem cell populations to replace lost neurons, and this may be the best option for the future.

The main research focus, however, has been in understanding how to proliferate and differentiate embryonic stem cells. Stem cells can be grown in large numbers from embryonic tissue, from embryonic stem cell lines and to a lesser extent from explants of adult CNS. A particular focus of this work has been the production of the dopaminergic neurons of the substantia nigra, which may be useful for implantation in the treatment of Parkinson's disease and the production of motor neurons for the

treatment of motor neuron disease. The key has been the identification of secreted molecules such as sonic hedgehog that influence neuronal differentiation during development, and the finding of transcription factors that are turned on in neurons as they undergo final differentiation. Reproducing these influences has led to the production of large numbers of motor neurons and dopaminergic neurons.^{30,31} However, the experience of transplanting these cells into animal models of disease has not to date been very successful in functional terms. Transplanted stem cells integrate well when transplanted into the regions of the brain that normally produce new cells, but in other brain regions transplant survival has generally not been good and the cells have integrated poorly into the CNS circuitry. Stem cell treatments for damaging conditions of the CNS will clearly be important in the future, but much basic research needs to be done to unravel the many complex issues before these treatments will be ready for clinical practice.

The future

One of the frustrations of the practice of clinical neurology has been the lack of treatments available to correct structural damage to the CNS. Basic research has now developed a number of treatments to enhance plasticity, axon regeneration and remyelination, and cell-based treatments will be developed in the next few years. Some of these treatments are in clinical trials, and others will follow soon. As these treatments enter clinical practice, the range of therapies available to neurologists and neurosurgeons will increase radically, as will the therapeutic possibilities. The coming decades will be an exciting time to be working in these fields.

Competing interests

James Fawcett is a paid consultant for Acorda Therapeutics Inc. which is developing chondroitinase for clinical use.

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