Neuroprotective Therapies for Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is considered to be an autoimmune disease that is caused by the immune system attacking the central nervous system (CNS) leading to myelin loss and axonal damage, resulting in long-term disability. The pathophysiology of MS is complex with involvement of genetic and environmental factors that define the susceptibility to generate the autoimmune attack. In the last decade, several immunomodulatory drugs have been approved, including recombinant proteins such as interferon-beta, monoclonal antibodies such as natalizumab, or small chemicals including glatiramer acetate. In addition, there is a wide pipeline of new immunomodulators finishing Phase II or III trials. However, at present there are no approved treatments that directly reduce nervous system damage or enhance its repair. Novel neuroprotective agents have been identified in pre-clinical studies but their development is being prevented by the absence of appropriate understanding of the mechanisms of CNS damage by inflammation as well as by the lack of clinical platforms to test them. In this review, we describe the different mechanisms of axonal injury and discuss some of the principal therapeutic candidates that could provide neuroprotection in MS.

Keywords

Multiple sclerosis, therapy, neuroprotection, regenerative therapies, neuroinflammation, neurodegeneration, demyelination, remyelination, axonal damage

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Multiple sclerosis (MS) is not only an inflammatory demyelinating disease as classically described but also a neurodegenerative disease with significant axonal loss, affecting all regions of the central nervous system (CNS). MS patients show significant axonal loss and to less extent neuronal loss in the grey matter, as well as multi-focal demyelination, oligodendrocyte loss and grey and white atrophy.[1,2] Current immunomodulatory therapies mainly treat the inflammatory component of the disease, however they may partially confer indirect neuroprotection due to prevention of the inflammatory damage that can produce degenerative changes in the long-term. Axonal and neuronal injury in MS occurs early in the disease course with damaged axons detected in histological specimens during the first year of diagnosis.[3–6] The presence of damaged axons from the early stages of the disease has raised the current concept of axonal pathology in MS as the cumulative result of inflammatory events and emphasises the need for early neuroprotective intervention.

The term neuroprotection is not well defined, but it is understood as the activation of a number of processes essential to neuronal survival, differentiation and functioning.[7] A neuroprotective therapy is the one with a beneficial effect in preserving the nervous system tissue and function against neurodegenerative diseases or brain injury. This effect may take the form of protecting neurons from apoptosis or degeneration and must not only target the pathogenic mechanism inducing tissue damage (e.g. restoring blood flow in stroke or preventing inflammation in MS). In the case of MS and considering that immunomodulation has achieved a significant control of the autoimmune process, now the current challenge is the development of neuroprotective and regenerative therapies[8]. This is critical for several reasons. First, immunomodulatory drugs may induce significant side effects, which are related with the level of immunomodulation achieved, limiting its dosage and therefore, its efficacy. For this reason, it is highly probable that some degree of residual inflammatory activity would remains, inducing tissue damage (immunopathology). Second, current immunomodulatory drugs target mainly the activity of the adaptive immune system, without preventing to significant extent the pro-inflammatory activity of resident microglia. Chronic microglia activation is present in all stages of the disease and it has been associated with axonal damage.[9,10] For both reasons, in the near future is highly likely that even in presence of a good battery of highly efficacious immunomodulatory drugs, a certain degree of chronic inflammation within the CNS will remain, requiring neuroprotective strategies. Moreover, although a significant proportion of axonal and myelin loss is due to the acute inflammatory damage, imaging and pathological studies have shown that brain atrophy and axonal loss progress along time, indicating the presence of degenerative process.[11,12] The mechanisms of this degenerative component are not well understood but it has been suggested that the lack of support of myelin to axon metabolism and function and the presence of glial scars challenge long-term axon survival.[13,14] This long-term axonal damage seems to be in the basis of the progressive phase of MS and

Table 1: Neuroprotective Therapies by Categories, Mechanism of Action and Developmental Status for Multiple Sclerosis

Category	Therapy	Mechanism of Action	Status
Channel blocking agents	Phenitoin, Lamotrigine	Sodium channel blockers	Phase II–III: no efficacy
	Bepridil	Calcium channel blockers	Preclinical
	4-aminopyridine (Dalframpidine)	Potassium channel blockers	Approved
Glutamate receptors antagonists	Memantine, riluzole, flupirtine	NMDA partial antagonist, glutamate release inhibition	Phase II: no efficacy
Trophic factors	NGF, BDNF	Trophic signals through Neurotrophin pathway	Preclinical
	IGF-1	Trophic signals through IGF pathway	Phase II: no efficacy
	EPO	Trophic signals through EPO pathway	Phase III
	LIF, CNTF	Trophic signals through Neurocytokine pathway	Preclinical
Cannabinoids	Nabiximols	Activation CB1 and CB2 receptors	Approved
MMP inhibitors	Lipic acid, BBI minocycline	Prevention of neuronal damage by MMPs	Preclinical, Phase III
Metabolites	Kynurenines	Modulation tryptophan pathway	Preclinical
	Fumarate	Modulation Nerf2 and anti-oxidant pathway	Phase III
	Polyamines and methyltioadenosine	Modulation cell cycle and cell survival	Preclinical
	Quercepin	Flavonoid pathway	Preclinical
Stem cells	Mesenchymal	Immunomodulatory and neuroprotective	Phase II
	Oligodendrocyte	Remyelination	Preclinical
	Neuronal	Neuronal repair?	Preclinical
Hormones	Triiodothyronine (T3)	Trophic factor activity on oligodendrocytes	Preclinical
	Steroids	Trophic factor activity on oligodendrocytes	Phase II
Pro-remyelination	Olexosime	Oligodendrocyte maturation	Phase III
Monoclonal antibodies	Anti-Lingo	Antagonist of inhibitor receptor Lingo	Phase II

Status refer to the level in the pre-clinical and clinical development process for treating patients with multiple sclerosis. BBI = Bowman-Birk inhibitor; BDNF = brain-derived neurotrophic factor; CNTF = ciliary neurotrophic factor; EPO = erythropoietin; IGF = insulin-like growth factor; LIF = leukaemia inhibitory factor; MMPs = matrix metalloproteinases; NGF = nerve growth factor; NMDA = N-methyl-D-aspartate.

the accumulation of disability. For the overall mentioned reasons, development of new therapies protecting myelin and axons against damage induced by inflammation and the degenerative process is now the first priority in MS therapy research.

MS may offer a good opportunity for providing a proof of concept of neuroprotection as a useful approach for treating brain diseases, compared to previous and many times disappointing, experiences in stroke, Alzheimer disease (AD) or brain trauma. In MS several of the immune mechanism of damage implicated have been identified, which contrast with other degenerative diseases in which pathogenesis remains largely unknown. Second, at the time of the diagnosis, MS patients have low degree of CNS damage and because they are young (in their 30s to 40s) they still have significant CNS self-repair capability, providing a window for protecting the tissue against future damage. This again is in contrast with other degenerative diseases in which patients are older and they suffer other co-morbidities that impact the homeostasis of the CNS and its self-repair capability. Also, at the time of diagnosis, in other neurodegenerative diseases such as AD or Parkinson's disease (PD) a significant proportion of neurons (more than half of total neuronal population) have already died and the rest may have been already damaged by the disease, giving a small window for intervention. Finally, one of the main limitations in the development of new neuroprotective therapies is how to measure the efficacy in clinical trials. The problem is that neurodegeneration is a slow process taking place along decades that poorly translate in the diagnostic tools available at present such as imaging or molecular biomarkers. The development of new imaging techniques for MS such as non-conventional magnetic resonance imaging (MRI), molecular imaging by positron emission tomography (PET) or optic coherent tomography (OCT) would give the opportunity to measure the effect of neuroprotective therapies with high resolution.[15–17]

Mechanisms of Axonal Injury

The mechanisms underlying axonal injury in MS are numerous and complex, being even difficult to distinguish between the acute axonal injury and the chronic injury processes.

Axonal Injury During the Acute Inflammatory Phase

Several studies have shown a direct correlation between axonal loss and acute lesions.[18,19] The evolution of active MS lesions involves loss of myelin, the presence of activated macrophages digesting myelin degradation products and T-cell infiltrate. CD8+ T-cells are normally present in these lesions and several studies reported the association between axonal damage and the presence of this cell type in the lesions giving evidence for T-cell mediated citotoxicity as a mechanism of acute axonal injury.[20,21] Glutamate is the main excitatory neurotransmitter, and over-excitation by glutamate has been implicated in acute axonal injury as well. Demyelinating axons over-express one type of glutamate receptors, the excitatory AMPA receptors and an excessive excitatory activation results in toxic accumulation of intracellular calcium and sodium leading to oligodendrocyte death.[22] There is also an increase in glutamate levels after inflammation due to an increase of vesicular release from unmyelinated axons in whitematter[23] and dysfunction of sodium-dependent glutamate receptors.[24,25] Moreover, immune cells from patients with MS release glutamate that can damage oligodendrocytes.[26,27] Neurons synthesise nitric oxide (NO), which has been also implicated in axonal damage. NO plays a physiological role in the CNS as a potent signalling molecule and inhibits mitochondrial respiration leading to subsequent energy failure within the axon. Data from different groups also suggest that NO released from inflammatory cells is associated with axonal damage.[28,29] NO is overproduced in acute inflammatory lesions through an up-regulation of the inducible synthases (iNOS or NOS2).[30]

Progressive Axonal Injury in Chronic Plaques

Axonal loss within chronic MS plaques is highly variable but it may account for a reduction of 60–70 % in axonal density.[31] This axonal loss occurs also in normal appearing white matter (NAWM) away from plaques due to distant (Wallerian) tract degeneration.[32] As in the acute lesions, in the chronic active plaques axonal injury is associated with inflammation mainly induced by activated microglia.[33] Trophic factors produced by different cell types promote normal axon function and survival.[34–36] During demyelination there is a lack of trophic factors, which has been claimed as one of the mechanism for the failure of remyelination and axonal loss. As axonal injury in chronic plaques is likely to be mediated by multiple mechanisms, other possible mechanisms implicated are conduction defects due to sodium and calcium channel dysfunction. There are several studies showing the contribution of conduction defects along chronically demyelinated axons in association with the progression of neurological disability.[37,38] Axonal conduction is a continuous energy dependent process that relies on the presence of ion channels and is essential for maintenance of the nerve impulse. If demyelination is present, axonal conduction becomes disrupted and sodium channels, which are normally situated in the Nodes of Ranvier, require being re-distributed along the all axon membrane. This altered distribution pattern is thought to be the cause of persistent sodium current production along the demyelinated axon, which requires far more energy to restore electrical neutrality through the Na^+/k^+ ATPase pumps than in not damaged areas. Furthermore, alteration of the Na^+ and K^+ exchanger usually alters intracellular Ca^{2+} homeostasis. Dysfunction of the Na^+/Ca^+ exchanger in injured axons due to increased intracellular Na^+ subsequently causes increased intracellular calcium concentrations that lead to calcium-mediated degenerative responses.[39,40] All these changes normally lead to an increase in energy demand in the demyelinated axon, inhibition of mitochondrial function and finally, cellular death.[41] Finally, the lack of support of myelin to axon metabolism and functioning challenge long-term axon survival, especially for small axons and long pathways.[13,14]

Neuroprotective Therapeutic Strategies for Multiple Sclerosis

Channel Blocking Agents

Sodium Channel Blockers

The role of sodium channel blockers has gained importance since the recognition of the role of increased sodium permeability in axonal injury. Excessive sodium channel activation increases depolarisation, toxic levels of calcium, release of glutamate and excessive energy demand on the damaged axon.[41] In mice with experimental autoimmune encephalomyelitis (EAE) the treatment with the sodium channel blocker phenytoin was able to protect axonal integrity and to preserve neurological function.[42] However, a two-year randomised double-blind placebo-controlled Phase II trial with the sodium channel blocker lamotrigine, in 120 patients with secondary progressive MS (SPMS), showed no benefit on brain volume loss.[43] Nevertheless, further studies following this type of therapy are necessary in order to better understand the possibilities of sodium channel blockers in neuroprotection.

Calcium Channel Blockers

The capacity of calcium antagonists to ameliorate axonal loss in MS has been explored in a number of *in vitro* and animal studies. In a study of EAE, bepridil, a broad-spectrum calcium channel blocker, prevented axonal loss and disability in treated animals.[44] There is however no current clinical trials of calcium channel blockers in patients with MS.

Potassium channel blockers: when axons are demyelinated they expose their voltage-gated potassium channels normally just located in the intermodal regions impairing action potential propagation. A study made with 4-aminopyridine, a potassium channel blocker, shows that this molecule could reverse conduction defects in demyelinated nerves.[45] In 2010, dalfampridine, an orally administered extended release form of 4-aminopyridine, was approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for improving walking speed in MS. Therefore, current indication is as symptomatic therapy and is still required to probed whether dalframpridine has neuroprotective effects in the long term.

Glutamate Receptor Antagonists

Glutamate contributes to axonal damage and there are several studies demonstrating the neuroprotective effects of AMPA (α-amino-3-hydroxy-5-methil-4-isoxazol-propionic acid) receptor antagonists such as NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo quinoxaline) in animals.[46,47] The protective effects of glutamate receptor antagonists are especially important because AMPA/kainate receptors become the primary mediators of axonal injury during prolonged injury exposure in the chronic phase of the disease.[48] However, the partial antagonist of NMDA receptor memantine showed transient clinical pseudo exacerbation in a Phase II trial in patients with MS, which significantly limits its use in patients with MS.[49] Riluzole, an inhibitor of the glutamate release from nerve terminals that also modulates the activities of both kainite and NMDA receptors approved for amyotrophic lateral sclerosis (ALS), was assessed in a small cohort of primary progressive MS (PPMS) patients. The study revealed a non-significant reduction in the rate of cervical cord atrophy and decrease in the development of T1 hypointense lesions but further studies increasing the population and different MS subtypes should be done.[50] Also, Flupirtine, an NMDA antagonist with anti-pain effects, has shown beneficial effects in the EAE model.[51]

Growth Factors

Growth or trophic factors are small proteins released by target cells that deliver prosurvival signals to the cell promoting proliferation or cell differentiation. There is a complex network of trophic factors that works in different combinations and cell types regulating development and tissue homeostasis. Due to its fundamental role in neuronal survival and myelination, they have been explored as prime candidates for neuroprotection in many neurological diseases.

Neurotrophins

Nerve growth factor (NGF) is a trophic factor for cholinergic, sympathetic and sensory neurons as well as for regulating the myelination process, which is also released in response to CNS damage.[52] Studies in the animal model of MS have shown that NGF modulates brain inflammation and promotes myelin and axonal preservation.[53] Brain derived nerve factor (BDNF) is another neurotrophin regulating the survival of several different populations in the brain and it has been implicated in the modulation of log-term memory or in the pathogenesis of depression or AD as some examples. For this reason it offers the promise as neuroprotective agent.[54] Moreover, lymphocytes within the MS plaques are able to release BDNF, which is used as an example of the protective effect of natural autoimmunity. To date, none of them has been tested yet in clinical studies in patients with MS. However, they have been tested in several Phase II and III trials in AD, PD, ALS or neuropathies. Results are not conclusive because presence of side effects limited the dosage.

Insulin-like Growth Factor-1

The possibility to facilitate remyelination by promoting oligodendrocyte function through the use of growth factors such as insulin-like growth factor-1 (IGF-1) has been explored in several studies. Reports of the effect of IGF-1 in EAE models are conflicting with some of them showing an improvement in disability, others showing just a transient effect and a more recent study failed to show any improvement.[55–57] A pilot study in just seven patients with SPMS failed to show improvement in the primary MRI endpoints.[58]

Erythropoietin

Erythropoietin (Epo) is a haemapoietic growth factor that, together with its receptor (EpoR), is widely expressed in the CNS with beneficial effects on different models of neurological injury.[59] Studies in the EAE animal model have shown anti-inflammatory and neuroprotective effect of Epo with less axonal loss in the treated group in comparison with controls.[60] A pilot study of human recombinant Epo in eight MS patients (SPMS and PPMS) reported clinical improvement of motor function and improved cognitive performance. However, there was no change in the MRI measures.[61] A larger, randomised-controlled study is in progress.

Neuropoietic Cytokines

Several studies suggest that leukaemia-inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) enhance neuronal survival when axonal injury is present. For example, local administration of recombinant CNTF prevented neuronal degeneration in an axotomy model.[62] More recent works suggested that CNTF neuroprotective effect could be indirect. Krady and colleagues showed that CNTF-stimulated microglia *in vitro* enhanced the survival of spinal cord motor neurons[63] and similarly, the conditioned medium from CNTF-stimulated astrocytes also was able to rescue motor neurons.[64] Nevertheless, CNTF has also been shown to promote neurite outgrowth and neuron survival in dorsal root ganglion neurons *in vitro*.[65] In the motor cortex of MS patients an up-regulation of the CNTF signalling pathway (increased expression of CNTF-receptor-α, LIF–receptor-β, gp130 and CNTF) has been shown as a compensatory response to disease pathogenesis in these patients.[66] However, they have not been tested in clinical trials yet.

Cannabinoids

There are *in vitro* evidences supporting the effect of cannabinoids on several mechanisms of axonal injury, such as, for example, glutamate release,[67] oxidative free radicals and damaging calcium flux.[68] In the EAE animal model, exogenous agonists of the cannabinoid CB1 and CB2 receptors showed neuroprotective effects.[69,70] Recently, a cannabinoid extract (Nabiximols) composed by tetrahidrocannabinol y cannabidiol has been approved as a symptomatic therapy for MS for improving spasticity and pain. However, despite the numerous works supporting the neuroprotective effects of cannabinoids, this beneficial effect remains to be proved in clinical trials.

Matrix Metalloproteinases Inhibitors

The matrix metalloproteinases (MMPs) regulate the infiltration of leukocytes across the blood–brain barrier (BBB) into the CNS. MMPs can also be neurotoxic, destroying neurons in tissue culture[71] thus inhibitors of these molecules have potential utility as neuroprotective agents in MS. Several inhibitors of MMPs have shown beneficial effects in the animal model of MS such as lipic acid, the Bowman-Birk Inhibitor.[72,73] The tetracycline derivative minocycline has shown anti-inflammatory and antiapoptotic activities. It is also an inhibitor of MMP activity. In a small trial of patients with relapsing-remitting MS (RRMS), it was determined that minocycline resolved gadolinium-enhancing MRI activity over three years corresponding to the reduction of proinflammatory molecules and MMP-9 in the sera of the treated patients.[74–76] Minocycline is currently in a Phase 3 trial of clinically isolated syndrome (CIS) to determine whether conversion to MS could be reduced.

Metabolites

Kynurenines

The synthesis of the metabolites kynurenic acid (KYNA), 3–hydroxykynurenine, quinolinic acid and others is produced through the kynurenine pathway, which is under a tight control of the immune system. An impaired control of this pathway is directly associated with neurological disorders and neurodegeneration. In the last few years, numerous agents have been developed for the study of this kynurenine pathway. Kynurenine aminotransferase II inhibitors (KAT II-inhibitors) intracerebrally applied caused a decrease of 30–40 % in extracellular KYNA, indicating that KAT II is responsible to control only a part of the KYNA synthesis but still enough to increase extracellular levels of glutamate, dopamine and acetylcholine. These effects result in pro-cognitive effects and attenuation of ketamine-induced working memory deficits in monkeys. Kynurenine-3-monooxygenase (KMO) inhibitors showed anticonvulsant and neuroprotective effects in various model systems *in vivo*.[77]

Fumarate

BG00012 is an oral formulation of dimethylfumarate that has demonstrated beneficial effects on relapse rate and magnetic resonance imaging markers of inflammation and axonal destruction in a Phase II and III MS trial. Dimethylfumarate is able to activate nuclear factor (erythroid-derived 2)-related factor 2 (Nerf2), increasing cytoprotection that augments the natural response against oxidative stress. This effect allows the preservation of myelin and axons in chronic autoimmune demyelination and also reduces loss of CNS neurons in EAE.[78]

Polyamines

The endogenous polyamines play different roles in a variety of cell functioning aspects. Polyamines may even be involved in brain ischaemia through modulation of potassium affecting ion channels and receptors, modulating calcium trafficking or through the production of toxic metabolites. The polyamine antagonists, ifenprodil and eliprodil have neuroprotective potential, although the mechanism of action is still under further study.[79] Its downstream metabolite, methylthioadenosine (MTA), has also been found to prevent acute EAE and reverse chronic-relapsing EAE inhibiting brain inflammation and damage.[80] We have recently observed that MTA displays a wide range of neuroprotective activities against different insults. MTA was also able to reduce axonal loss and protect neurons from death in different neurodegenerative animal models (unpublished data).

Quercetin

Quercetin is one of the most studied flavonoids showing beneficial effects in different diseases such as cardiovascular and inflammatory disorders and cancer therapy. In the last few years, several publications show up with the effects of this molecule in neuroprotection. Although there are several positive results showing its direct action on neurons and in various animal models of neurodegeneration or neurotoxicity, there are also some concerns about using quercetin as a neuroprotective compound. First, after oral administration, it is not possible to detect the molecule either in plasma or in the brain. Second,

some of the most effective quercetin metabolites are not able to cross the BBB and third, the therapeutic dose-range is very narrow.[81]

Strategies for Promoting Remyelination

Pre-clinical research has identified several compounds to promote remyelination but at present none of them have been approved. There are two major approaches being tested in the animal models: cell replacement by cell transplantation (exogenous approach) and promotion of repair by resident stem and precursor cell populations in the adult CNS (endogenous approach). It is important to take into account that the efficacy of any remyelinating therapy depends directly on the ability to suppress the effect of ongoing disease process affecting new oligodendrocytes and myelin. In MS, this issue can be a major concern, but the application of effective anti-inflammatory drugs may provide the suppressive environment in which remyelination could be effective.

Enhancing Remyelination by Cell Therapy

The use of cell transplantation to promote remyelination comes from the early 1980s and many studies have used this approach with different cell types. The advantage of myelin cell replacement compared with other types of stem cell therapies is that myelin cells are not specific for a given brain region and they are not fixed in the wiring of neuronal connexions like neurons are, which gives them the opportunity to be replaced. Moreover, the CNS contemplates the possibility of forming new oligodendrocytes and myelin sheaths from oligodendrocyte precursor cells (OPCs) as part of the repair mechanism, which means that the brain do not impose significant barriers for oligodendrocyte replacement compared with neurons, which are post-mitotic and the presence of neuron progenitors are very restricted to a few specific areas of the CNS. Also, remyelination is contemplated as one of the most important mechanisms of neuroprotection, because chronic axonal injury seems to be dependent of the failure of axons to survival without the support of myelin.[13,14] In order to develop cell therapy for enhancing remyelination, there are some concerns that have to be addressed. First, why to transplant OPCs into lesions that already contain these cells and in an environment that is inhibiting differentiation and regeneration? A second concern is the delivery method. For focal lesions, single injections may provide a sufficient spread of cell for repair, however the most common scenario in MS will require multiple injection sites or the ability of cells to reach all damaged regions. Intracerebral administration is risky, so other experimental approaches have been developed such as intraventricular and intravenous delivery.[82,83] Third, the source of the cells is another limitation. There would be problems with the availability of some type of cells for human therapeutic applications. Stem cell lines represent a potential source of cells that can provide unlimited quantities of precursors, but nowadays with limited capacity of differentiation into oligodendrocytes.

Mesenchymal stem cell (MSC) transplantation has been the first attempt to translate stem cell research to clinical practice that has been effective. In EAE and other animal models of tissue injury and inflammation, MSCs have shown beneficial effects.[84,85] Several pilot Phase II studies of autologous MSC transplantation in patients MS have shown promising results, but the main mechanism of action for MSC is immunomodulation and may be some degree of neuroprotection, but true regenerative effects remains to be demonstrated.[86,87] Also, there are another ongoing pilot trials with oligodendrocyte stem cells with the aim of achieving the goal of remyelinated CNS lesions (NCT00283023).

Enhancing endogenous remyelination: in order to develop this type of therapy is important to understand the mechanism of remyelination and to identify therapeutic targets among these pathways. Remyelination failure may be associated with insufficient OPC recruitment, failed OPC/oligodendrocyte differentiation or failure of myelin prolongations to unsheathe axons. The mechanisms that directly promote OPC differentiation are the most active areas of research in order to develop therapies for enhancing remyelination. Of particular interest are chemokine CXCL12 and its receptor CXCR4 that mediate migration, proliferation and differentiation of neuronal precursors within the developing CNS.[88] Another receptor found to promote oligodendrocyte formation and maturation is the thyroid hormone beta receptor.[89] As such, Triiodothyronine has shown in animal models of MS the capacity of promoting remyelination and decreasing disability in the primate model.[90] Of course, other compounds that are not endogenous to the system but can enhance the function of OPCs have also been found. As several growth factors have been shown to promote OPCs survival and proliferation, some studies use this fact to develop drugs that could replicate these actions. One example is olesoxime, a cholesterol-oxime compound, which in a dose dependent manner is able to accelerate OPC differentiation by promoting their maturation *in vitro*. This compound shows promise as a therapy for MS because is orally bioavailable, crosses the BBB and is safe for humans.[91] Another example of a molecule that has been found to have an effect on OPC differentiation is minocycline. Minocycline was able to decrease the severity and progression of EAE in mice. In other *in vitro* models, minocycline showed promotion of remyelination via immature oligodendrocyte differentiation by weakening microglial reactivity.[92] Finally, the first drug being developed at the clinical level for enhancing remyelination is anti-Lingo mAb. Lingo is an inhibitory receptor of myelination and targeting Lingo expression has shown enhanced remyelination after demyelination in animal models.[93] Anti-Lingo is now entering Phase II trials in patients with RRMS and acute optic neuritis.

Role of Immunomodulatory Therapies in Tissue Protection

Immunomodulatory therapy reduces the number of pro-inflammatory cells and immune mediators in the CNS by different mechanisms and therefore, the propensity for injury by these cells. For this reason, even if they are not neuroprotective drugs, they can account for a proportion of the prevention of tissue damage. Accordingly, immunomodulatory drugs may reduce brain atrophy progression in MS even if they do not cross the BBB.[94–97] Natalizumab treatment has shown to reduce brain atrophy in clinical trials, possibly as a result of the significant blockage of the BBB passage by pro-inflammatory cells. Similarly, monoclonal antibodies that are able to deplete specific subsets of immune cells, like alemtuzumab, rituximab, ocrelizumab or daclizumab have also lowered brain atrophy in MS.[98] Data from first line immunomodulators like glatiramer acetate (GA) and interferon beta (IFNβ) also show reduction in brain atrophy.[97,99] Fingolimod is a sphingosine 1-phosphate receptor modulator that blocks lymphocyte trafficking by trapping lymphocytes in lymph nodes reducing their circulation to peripheral inflammatory tissues. This treatment has recently been shown to have also a favourable impact in oligodendroglial progenitor cell (OPC) homeostasis and remyelination processes by modulation of the SIP5 receptor *in vitro*.[36] However, whether fingolimod exerts neuroprotective effects in patients still needs to be demonstrated. Laquinimod is an immunomodulatory agent that is being developed for the treatment of RRMS. A recent

study shows a direct and sustained effect of laquinimod on the up-regulation of BDNF in patients with RRMS. This finding was also verified in the EAE model using a conditional BDNF knockout strain.[100] Finally, another immunomodulatory drug being developed for RRMS is dimethyl-fumaric acid (BG-12). BG-12 has a complex mechanism of action, activating Nerf 2, resulting in anti-inflammatory and anti-oxidant effects.[78] The neuroprotective activity of BG-12 requires further exploration in clinical trials.

The Future of Neuroprotection in Multiple Sclerosis

It is clear that neurodegeneration is an important process in MS and it is therefore crucial to achieve better results in preventing CNS damage with neuroprotective strategies. First, we need therapies in order to prevent or reduce the destruction of neurons and axons more effectively. This type of treatment would provide a beneficial effect directed at neurons and glial cells within the CNS, particularly medications able to activate programmed axonal loss or neuronal death. Another possible approach is the activation of astrocytes, which provide protective molecules for axons and neurons like trophic factors and metabolic balance. Second, neuroprotective therapies should start early in the course of the disease, when tissue damage is still not extensive, giving a window for protecting axons and myelin against damage. Axonal pathology is present from disease onset[6] and significant axonal degeneration often follows an acute relapse which enhances disability progression,[9] indicatingthat neuroprotective therapies should be started in early RRMS and in combination with the current standard of care drugs, the immunomodulatory drugs. Third, combination therapy with immunomodulatory and neuroprotective drugs may provide additional benefits, by providing a multi-faceted approach to MS pathogenesis, targeting the detrimental inflammatory processes and also providing CNS cells elements to resist inflammatory and non-inflammatory insults.

Several agents with neuroprotective activity have been shown to be protective in animal models and the challenge now is to translate promising pre-clinical candidates into meaningful designed trials in humans. In order to achieve such a goal, the development of more predictive models on CNS damage, new biomarkers and clinical tools (e.g. computational classifiers) for assessing the neuroprotective effects in patients with MS is required.[16,101] Anti-inflammatory agents will continue to represent a major focus for MS therapy, however, combination therapy, with neuroprotective agents, is starting to show great potential in minimising disability in MS. ■

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