Neuroprotection and Recovery in Multiple Sclerosis

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Abstract

Multiple sclerosis is a complex and heterogeneous immune-mediated disease that results in the progressive accumulation of mental and physical symptoms. Currently approved disease-modifying drugs (DMDs) are immunomodulatory or immunosuppressive, but these drugs have little effect on disease progression. In addition to studies that have directly targeted inflammation and immune responses, a large number of studies, most of them experimental, have investigated neuroprotective therapies and remyelination strategies. However, to date, attempts to provide neuroprotection have failed not just in multiple sclerosis but in neurological disorders in general; this situation has emphasized the need to revise the old paradigm of a "magic bullet" with a single mechanism of action. Remyelination strategies involve either promoting endogenous remyelination or replacing lost myelinating cells through exogenous sources. However, several puzzle pieces regarding the physiology of remyelination remain unknown, including feasible treatment monitoring methods, the selection of patients, and the optimal time of treatment initiation. This chapter will describe the direct and indirect neuroprotective effects of DMDs, as suggested by basic research studies and confirmed by clinical studies in some cases. Current knowledge of potential neuroprotective therapies and remyelination strategies is also reviewed.

Keywords: multiple sclerosis, neuroprotection, ion channel modulation, remyelination, systems biology

1. Introduction

Multiple sclerosis (MS) is characterized by complex interactions between pathological pathways and heterogeneity regarding lesions, progression, clinical symptoms, and immune responses.

Recently, significant advances in MS therapy have been made, but these advances have been limited to the prevention of relapse, and long-term results are conflicting.

Understanding of endogenous defense activity (**Figure 1**), including neurotrophicity, neuroprotection, neuroplasticity, neurogenesis, and remyelination, is essential for pharmacological neuroprotection and enhanced neurorecovery. Neurotrophicity includes the processes necessary for the maintenance of a normal phenotype. Neuroprotection is the sum of all processes aimed at counterbalancing the pathophysiological mechanisms that are induced by the alteration of neuro-immune responses. Neuroplasticity represents the sum of the structural and functional changes that must occur for adaptation to new internal or environmental stimuli. Neurogenesis, in a broad sense, refers to the capacity of brain tissue to generate new neurons, astrocytes, and oligodendrocytes [1]. Remyelination is a physiological regenerative process that requires the activation of oligodendrocyte precursor cells (OPCs), their migration, recruitment, and differentiation into remyelinating oligodendrocytes and their interaction with denuded axons. Changes in these steps, which are characteristic of MS, promote neurodegeneration.
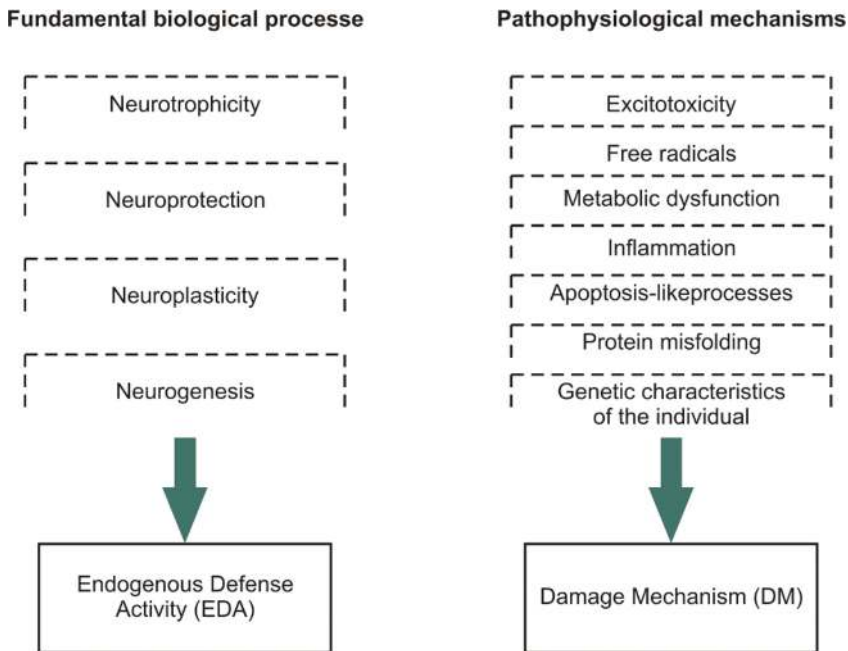

Figure 1. Endogenous defense activity and damage mechanism.

Classical neuroprotection approaches include the use of the already Food and Drug Administration (FDA)-approved disease modifying drugs (DMDs) and a wide spectrum of pharmacological compounds that interact with one or more pathological processes (inflammation,

oxidative damage, mitochondrial damage, and intracellular Ca^{2+} overload), as an attempt to prevent axonal degeneration. Pro-myelination therapies appear to be a promising approach, but several puzzle pieces regarding the physiology of remyelination, feasible treatment monitoring methods, the selection of patients, and the optimal time of treatment initiation remain unknown. However, neurodegeneration is not always related to demyelination, leading to the development of combination therapies that include agents that prevent neurodegeneration, modulate neuroinflammation, and immune responses and promote remyelination [2].

2. Neuroprotective effects of disease modifying drugs (DMDs)

Several DMDs are currently approved by the FDA for MS: interferons (interferon beta 1b or IFNB-1b, interferon beta-1a or IFNB-1a), glatiramer acetate (GA), traditional immunosuppressants (mitoxantrone), fingolimod, and monoclonal antibodies (natalizumab, alemtuzumab, and daclizumab) as well the recently approved drugs teriflunomide and dimethyl fumarate (DMF). The main target of these molecules is the modulation of immune mechanisms and inflammation, along with a debatable effect on disease progression. **Table 1** summarizes the available information about FDA-approved DMDs, including their mechanisms of action and severe adverse effects [**Table 1**]. The neuroprotective effects of these agents against neurodegeneration and their ability to promote reparative processes are still under investigation.

FDA-approved DMDs	Indication	Primary mechanisms of action	Neuroprotective effects—results from basic research studies	Neuroprotective effects—results from clinical research studies	Severe adverse effects
Interferon beta-1b (Betaseron, Extavia)	First line therapy for RR-MS, SP-MS, and CIS	Suppresses the proliferation of MBP-specific T cells. Inhibits the secretion of pro-inflammatory cytokines	Stabilizes BBB barrier. Protect endothelial cells from apoptosis Decrease the expression of matrix metalloproteinases. Anti-inflammatory effects. Antioxidative and anti-excitotoxic effect. Increase BDNF and NGF levels [3–5]	Higher serum levels of BDNF in patients treated with IFNβ [6–8] Reduces the likelihood of the development of black holes and reduces the size of pre-existing ones [9]	Hepatotoxicity, congestive heart failure, seizures, depression or suicidal thoughts

FDA-approved DMDs	Indication	Primary mechanisms of action	Neuroprotective effects—results from basic research studies	Neuroprotective effects—results from clinical research studies	Severe adverse effects
//5Interferon beta-1a (Avonex; Rebif)	First line therapy for RR-MS, SP-MS, and CIS (only Avonex)	Suppresses the proliferation of MBP-specific T cells. Inhibits the secretion of pro-inflammatory cytokines	–	–	Hepatotoxicity, congestive heart failure, seizures, depression or suicidal thoughts
Peg interferon beta-1a (Plegridy)	First-line therapy for RR-MS	Suppresses the proliferation of MBP-specific T cells. Inhibits the secretion of pro-inflamma-tory cytokines	–	–	Hepatotoxicity, congestive heart failure, seizures, depression or suicidal thoughts
Glatiramer acetate (Copaxone)	First-line therapy for RR-MS and CIS	Suppresses the proliferation of MBP-specific T cells. Shifts the population of T cells from proinflammatory Th1 cells to regulatory Th2 cells	Anti-inflammatory, antioxidative, and anti-apoptotic effects [10, 11]. Increased BDNF and IGF-2 Pro-remyelination and pro-regenerative proprieties [12, 13]	Conflicting results: there found both increased and no effect upon serum BDNF levels [14–16]. Imaging data supports the neuroprotective and pro-myelinating properties of GA by showing that patients treated with GA are less likely to develop "black holes" than non-treated patients and have demonstrated a significant increase in the NAA–Cr ratio compared to pre-treatment values	Injection site lipoatrophy and necrosis, panic disorder, bowel disorder

FDA-approved DMDs	Indication	Primary mechanisms of action	Neuroprotective effects—results from basic research studies	Neuroprotective effects—results from clinical research studies	Severe adverse effects
				[17]	
Mitoxantrone (Novantrone)	Third-line therapy for SP-MS, and worsening RR-MS	Suppresses the proliferation of T cells, B cells, and macrophages. Enhances T-cell suppressor function and inhibits B-cell function and antibody production. Inhibits macrophage-mediated myelin degradation	–	–	Secondary acute myelogenous leukemia, cardiotoxicity
Fingolimod (Gilenya)	First- or second-line therapy for RR-MS and SP-MS	Sequesters lymphocytes in lymph nodes	Promotes oligodentrocyte extension Increases BDNF and GDNF production [18]	–	Macular edema, bradyarrhythmia, PML, hypotension, herpes infection, hepatotoxicity
Natalizumab (Tysabri)	Second- or third line therapy for RR-MS	Inhibits leukocytes migration	–		PML, allergic reactions including anaphylactic shock, infections, hepatotoxicity
Daclizumab (Zinbryta; Zenepax)	Second line therapy for RR-MS	Inhibits the activation of T cells and inhibits survival of already activated T cells; inhibits secretion of pro-inflammatory cytokines. Normalizes the number of circulating LTi cells	–		Infections, cutaneous events, malignancies, auto-immunity

FDA-approved DMDs	Indication	Primary mechanisms of action	Neuroprotective effects—results from basic research studies	Neuroprotective effects—results from clinical research studies	Severe adverse effects
Teriflunomide (Aubagio)	First-line therapy for RR-MS	Inhibits the activation and proliferation of stimulated lymphocytes	–	–	Hepatotoxicity, peripheral neuropathy, hyperkalemia, transient acute renal failure, severe skin reactions
Dimethyl fumarate (Tecfidera)	First line therapy for RR-MS	Reduce transendothelial migration of activated leukocytes	Antioxidative effects by activation of Nrf2 [19, 20]	–	Lymphopenia
Alemtuzumab (Lemtrada)	Second line therapy for RR-MS	Lymphocyte B and T depletion; decrease of pro-inflammatory cytokines	Anti-inflammatory effects Induction of neurotrophin producing lymphocytes Preservation of axonal conductance Stabilizes blood–brain barrier [21]	It significantly decreases the T2-weighted lesion burden compared to IFNβ [22]	Infusion-associated reactions, infections, auto-immunity

Abbreviations: LTi—lymphoid tissue inducer; IGF-2—insulin growth factor; MPB—myelin-basic protein; BDNF—brain-derived neurotrophic factor; GDNF—glial cell-derived nerve factor; Nrf2—nuclear factor erythroid 2-related factor; BBB—blood–brain barrier, RR-MS—relapse remitting MS; SP-MM—secondary progressive MS, CIS—clinical-isolated syndrome; PML—progressive multifocal leukoencephalopathy; LTi—lymphoid tissue inducer.

Table 1. The neuroprotective effects of FDA-approved DMD.

In addition to the currently FDA-approved DMDs, some promising new agents are already in ongoing late-phase clinical trials, such as laquinomid, ozanimod, ponesimod, siponimod, ocrelizumab, ofatumumab, masitinib, and cladribine. Few data related to the mechanisms of action of these drugs are currently available. Of these compounds, laquinimod is the only one that appears to have neuroprotective properties, and laquinimod is currently being tested in patients with RR-MS in a third phase III trial, CONCERTO [23]. Basic research studies suggest that in addition to its neuromodulatory and anti-inflammatory effects, laquinimod also displays neuroprotective effects through several mechanisms, including reducing excitotox-

icity, increasing serum levels of BDNF, downregulating the astrocytic pro-inflammatory response, reducing astrocytic nuclear factor κB (NFκB) activity, and preserving cannabinoid receptor type 1 expression [24]. However, to date, the results of phase II and III clinical trials have failed to show a clear effect of laquinimod in RR-MS patients [25, 26].

3. Other neuroprotective strategies

In addition to DMDs, there are many additional potential neuroprotective agents, including ion channel modulators, glutamate antagonists, growth factors, sex hormones, statins, and immunophilin ligands. Most of these were tested only in experimental studies as a means to target molecular pathways involved in neurodegeneration or, in contrast, to stimulate endogenous defense mechanisms. There is increasing interest in pleiotropic molecules such as 5-HTR3 antagonists [27], polymerized nano-curcumin [28], and tyrphostin AG 126 [29]; in molecules that can modulate the kynurenine pathway [30]; in cannabinoid compounds [31–33]; and in combination therapies of DMDs with pleiotropic molecules.

One of the factors that contributes to the persistence of inflammation in MS is sustained activation of the transcription of nuclear factor kappa B (NFκB), which is an important hub for several molecular mechanisms involved in apoptosis and in immune and inflammatory responses. Glucocorticoid-induced leucine zipper (GILZ) is a glucocorticoid-responsive protein that binds the p65 unit of NFκB and thus can reduce the immuno-inflammatory response. In cell cultures, a synthetic peptide (GILZ-P) derived from the proline-rich region of GILZ suppressed NFκB activation and prevented glutamate neurotoxicity [34]. Additionally, in an in vitro study, intraperitoneal administration of GILZ-P modulated the Th1/Th2 balance and ameliorated the symptomatology of experimental autoimmune encephalomyelitis (EAE) [35]. The paracaspase mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) is another signaling molecule that triggers lymphocyte activation through NFκB signaling and also acts as a cysteine protease. To test the hypothesis that MALT1 inhibitors could be used to treat lymphocyte-mediated pathologies, the therapeutic potential of mepazine (a recently identified MALT1 inhibitor) was studied in mice with EAE. When mepazine was prophylactically administered, it significantly reduced clinical disease symptoms and histo-pathological parameters. Moreover, its therapeutic administration clearly promotes disease remission [36].

The nuclear receptor-related 1 protein (Nurr1) is a member of the class of steroid nuclear hormone receptors, and its activity is significantly downregulated in neurodegenerative disorders such as MS; its levels are also negatively correlated with EDSS progression. In mice with EAE, the administration of isoxazolo-pyridinone, an activator of the Nurr1 signaling pathway, delays EAE onset and reduces its severity. Therapeutic administration of isoxazolo-pyridinone also reduced neuro-inflammatory and histopathological alterations in the spinal cord but not the course of EAE [37].

KV1.3, the third member of the shaker-related subfamily of voltage-gated potassium channels, is known to modulate calcium signaling to induce T cell proliferation (effector memory T cells

—TEM), immune system activation and cytokine production. Toxins derived from animal venoms can target ion channels, including KV1.3, and offer a means to diminish the activation and proliferation of TEM cells and to improve of the pathology underlying autoimmune diseases. For example, in a rat acute EAE model, ADWX-1, an analog of scorpion toxin, reduced the number of T cells and the secretion of inflammatory factors. These toxic peptides could be used to obtain better clinical results without neurological impairment [38]. There is increasing interest in bee venom therapy, which experimental studies have shown can ameliorate the symptomatology of EAE by decreasing inflammation and demyelination [39]. However, additional clinical evidence is needed.

The mitochondrial permeability transition pore (PT pore) is a drug target for neurodegenerative conditions and for ischemia-reperfusion injury. Cyclophilin D (CypD) is a positive regulator of the pore, and its downregulation improves outcomes in animal models of stroke. However, this isomerase is not selective and may have toxic effects. A new synthesized mitochondria-targeting CypD inhibitor, JW47, displayed selective cellular inhibition and reduced cellular toxicity. In an EAE model, JW47 significantly protected axons and improved motor assessments with minimal immunosuppression. These findings suggest that selective CypD inhibition could become a viable therapeutic strategy for MS [40].

Granzyme B (GrB) is a serine protease released from the granules of cytotoxic T cells, which can induce cell death by disrupting a variety of intra/extracellular protein substrates. GrB-expressing T cells were identified in close proximity to oligodendrocytes and demyelinating axons in acute MS lesions and were thus associated with neuronal loss. The GrB inhibitor serpina3n, which was isolated from mouse Sertoli cells, can inhibit the enzymatic activity of this protease. The administration of serpina3n attenuated disease severity in an animal model of MS by reducing T cell-mediated neuronal death and axonal injury. These observations suggest that serpina3n could be used to decrease inflammation-mediated neurodegeneration [41].

Experimental studies have shown that fasudil—an inhibitor of Rho kinase (ROCK)—can suppress experimental EAE when administered via multiple, short-term injections. Later, a novel ROCK inhibitor that can be delivered intranasally was developed. This inhibitor, FSD-C10, reaches the CNS faster and in a much lower dose. FSD-C10 reduced EAE severity and CNS inflammatory infiltration and promoted neuroprotection by inducing CNS production of IL-10, NGF, and BDNF and by inhibiting the production of multiple pro-inflammatory cytokines [42].

Eriocalyxin B (EriB) is a diterpenoid extracted from Isodon eriocalyx, a perennial herb from southwest China that is used as an anti-inflammatory remedy in traditional Chinese medicine. EriB has been reported to induce apoptosis in leukemia and lymphoma by elevating the intracellular levels of reactive oxygen species and by suppressing the NFκB pathway. In an EAE model, EriB alleviated symptoms, delayed disease onset, decreased T cell populations, inhibited the NFκB pathway and reduced CNS inflammation and demyelination, improving the course of the disease [43]. Adenanthin, which is also a diterpenoid isolated from the leaves of Isodon adenanthus, displays preventive and therapeutic effects in EAE, as demonstrated

by improved clinical scores as well as by reduced infiltration of inflammatory cells and demyelination in the CNS [44, 45].

Regarding sex hormones, 2-methoxyestradiol (2ME2)—the endogenous metabolite of estradiol and an antimitotic and antiangiogenic cancer drug—was found to suppress the development of mouse EAE, as it inhibited lymphocyte activation, cytokine production, and proliferation in a dose-dependent manner [46]. Other studies have shown that estrogen and estrogen receptor agonists reduce the severity of EAE in animals when they are administered after disease onset; these agents inhibit several inflammatory cytokines, induce apoptosis in T cells, and also regulate the expression of adhesion and accessory molecules by endothelial cells, altering leukocyte migration [47]. In addition, the β estrogen receptor has been demonstrated to modulate microglial activity. The β estrogen receptor agonist LY3201 can suppress activated microglia and NFκB activation in both microglia and T cells. All of these outcomes can be achieved without negative effects on the pituitary gland, mammary glands, or uterus [48].

Nevertheless, in animal models of demyelination, progesterone and synthetic progestins have been observed to attenuate myelin loss and to reduce clinical symptom severity. One study showed that progesterone and Nosterone (a synthetic 19-nor-progesterone derivative) promoted remyelination and attenuated inflammatory responses in female mice with severe chronic demyelinating lesions. The remyelinating effect of progesterone was receptor-dependent and began in the corpus callosum. Moreover, it enhanced the number of mature oligodendrocytes and their progenitors as well, indicating that these hormones could represent promising therapeutic agents for demyelinating diseases [49].

Statins are widely used to treat vascular diseases, but they also have immunomodulatory and neuroprotective properties that could make them possible treatment candidates for neurodegenerative disorders. Lovastatin has been found to improve clinical symptoms associated with EAE as well as to reduce neuroinflammatory mediators such as iNOS, TNF-α and interferon gamma (IFNγ). Similarly, atorvastatin has also been shown to ameliorate EAE symptomatology by modulating T cell immunity [50]. One double-blind, controlled trial used simvastatin in patients with secondary progressive MS. High-dose simvastatin reduced the rate of whole-brain atrophy by 43% compared with placebo and was safe and well tolerated. Furthermore, differences between the simvastatin-treated and control groups were consistently observed over 12 and 25 months. A small but significant improvement in disability outcomes and a non-significant reduction in T2 lesion accumulation were also observed [51].

SWABIMS was a multi-center, randomized, parallel-group, rater-blinded study conducted in 8 Swiss hospitals that evaluated the efficacy, safety, and tolerability of daily administration of 40 mg atorvastatin and subcutaneous IFNB-1b compared to monotherapy with IFNB-1b. At the end of the study, both groups had an equivalent number of patients with new lesions on T2-weighted MRI images. Additionally, none of the secondary endpoints, including the number of new lesions and total lesion volume on T2-weighted images, the total number of new Gd-enhancing lesions on T1-weighted images, total brain volume, grey matter volume, white matter volume, EDSS, relapse rate and number of relapse-free patients, did showed any significant differences, suggesting that atorvastatin did not have a beneficial effect on relapsing-remitting MS [52].

Recent data from an established rat model of MS suggest that inhibiting excitatory glutamatergic neurotransmission may have neuroprotective effects. One of these studies investigated whether drugs such as amantadine and memantine (antagonists of NMDA glutamate receptors), LY 367385 (a selective mGluR1 antagonist) or MPEP (an mGluR5 antagonist) could improve the condition of rats with EAE. On the one hand, amantadine and memantine reduced the development and duration of neurological deficits and modified all of the assessed parameters. On the other hand, LY 367385 and MPEP did not influence the condition of treated animals when they were administered alone or in conjunction with NMDA antagonists [53]. Another study evaluated if selective antagonism of the NR2B subtype of NMDA receptors (which are considered to play a more pivotal role in neurodegeneration) could be more effective than memantine in EAE mice. Therapeutic administration of RO25-6981 (a selective inhibitor of NR2B) caused a more significant decrease in neurological deficits, inflammation, myelin degradation, and degeneration of axons from the spinal cord, suggesting that this drug may be an effective treatment strategy to slow down the clinical deterioration that causes disability in MS [54].

The metabotropic glutamate receptor 4 (mGluR4) has immunomodulatory properties, such that a positive allosteric modulator of the receptor, ADX88178, protects mice with relapsing-remitting EAE. ADX88178 is a newly developed drug with high selectivity and potency, optimal pharmacokinetics, good brain penetrance, and almost no toxicity. Its administration in EAE converted the disease into a form of mild chronic neuroinflammation that remained stable for two months after the drug treatment was discontinued [55].

Recent studies have demonstrated that atypical antipsychotic agents (antagonists of dopamine D2 and serotonin 5-HT2a receptors) have immunomodulatory properties, both peripherally and within the CNS. In an EAE animal model, chronic oral administration of risperidone improved disease severity, decreased both the size and the number of spinal cord lesions and substantially reduced antigen-specific interleukins such as IL-17a, IL-2, and IL-4 and the activation of microglia and macrophages in the CNS. In addition, another antipsychotic agent, clozapine, showed a similar ability to modify macrophages and to reduce disease severity. Together, these studies indicate that atypical agents could treat immune-mediated diseases such as MS [56].

Polyphenolic flavonoids and non-flavonoids have potent antioxidant abilities, but they can also target different molecules and affect multiple signaling pathways. Resveratrol, a phenol found in grapes and red wines, is considered to have neuroprotective effects. In EAE, it induces the apoptosis of activated T cells in the periphery and suppresses pro-inflammatory responses. Another plant-derived substance, oleanolic acid (a triterpenoid), is known to have potent anti-inflammatory properties. Treatment with oleanolic acid has been reported to prevent EAE by suppressing peripheral inflammation and preventing CNS infiltration of inflammatory cells (due to blockade of the NF-κB pathway [45]. Other studies have shown that flavonoids are naturally immunomodulatory compounds that can limit demyelination, reduce neuroinflammation, and downregulate immune functions. For example, luteolin provides neuroprotection by reducing axonal damage and, together with quercetin and fisetin, is able to decrease the amount of myelin phagocytosed by macrophages; thus, luteolin may help prevent MS [57].

Polyphenolic curcuminoids are the mixtures of curcumin, desmethoxycurcumin, and bisde-methoxycurcumin, which are derived from turmeric (*Curcuma longa*). Both the mixtures and the individual components have been suggested to influence inflammatory and apoptotic genes and the regulation of signal transduction pathways that lead to the activation of transcription factors. In EAE, treatment with curcumin modulates pro- and anti-inflammatory responses, prevents the differentiation of neural antigen-specific T cells, decreases oxidative stress, improves remyelination and promotes neurogenesis [28]. However, despite the promising therapeutic potential of curcumin, its poor water solubility, fast degradation profile and poor bioavailability are significant hurdles for its clinical use.

The kynurenine pathway is known to have a regulatory function in the immune system. Alterations of this pathway have been described in preclinical and clinical investigations of MS. These data led to the identification of potential therapeutic targets, such as synthetic tryptophan analogs, endogenous tryptophan metabolites, structural analogs, indoleamine-2, 3-dioxygenase inhibitors, and kynurenine-3-monooxygenase inhibitors [30]. Additionally, high levels of a by-product of the kynurenine pathway, quinolinic acid, were found in EAE mice and MS patients. Sundaram et al. demonstrated two possible strategies to limit quinolinic acid gliotoxicity: by neutralizing quinolinic acid's effects with monoclonal antibodies or by inhibiting quinolinic acid production using specific KP enzyme inhibitors. These observations could represent a novel therapeutic approach in MS [58].

Cannabidiol (CBD) is a non-psychotropic cannabinoid constituent of *Cannabis sativa* that is known to possess anti-inflammatory and immunosuppressive properties. In a viral model of MS, CBD decreased the transmigration of blood leukocytes by downregulating the expression of VCAM-1, chemokines and the cytokine IL-1β and by attenuating the activation of microglia. Its administration had long-lasting effects and ameliorated motor deficits during the chronic phase of the disease, demonstrating the significant therapeutic potential of this compound [59]. Another study of CBD as a topical 1% cream also had surprisingly good results too. The daily treatment, initiated at the time of symptomatic disease onset, displayed neuroprotective effects against EAE, diminishing clinical disease scores (EDSS) by recovering hind limb paralysis and by ameliorating lymphocytic infiltration and demyelination in spinal cord tissues [60]. However, when the CUPID trial investigated if oral dronabinol (Δ^9-tetrahydrocannabinol) might slow the course of progressive MS, it had no overall effect on disease progression, although there were no serious safety concerns [61].

Epigallocatechin-3-gallate (EGCG), one of the major polyphenolic extracts of green tea, has been shown to exhibit neuroprotective effects against toxic insults and neuronal injury. In an EAE animal model, the administration of EGCG attenuated clinical symptoms and leukocyte infiltration and demyelination in the CNS. Moreover, EGCG inhibited the NF-κB-mediated transactivation of inflammatory mediators, reducing the production of interferons, IL-17, IL-6, IL-1, and tumor necrosis factors [62]. These results were corroborated by other studies, which demonstrated that EGCG, due to its antioxidative properties, could reduce the clinical severity of EAE by limiting brain inflammation and reducing neuronal damage [63]. In addition, GA and EGCG combination therapy had synergistic protective effects in vitro and in vivo, with good results and no unexpected adverse events [64].

Ginseng has been used in traditional medicine for over 2000 years due to its antianxiety, antidepressant, and cognition-enhancing properties. Moreover, its effects on the brain are related to glutamatergic and monoaminergic transmission, estrogen signaling, nitric oxide production, neuronal survival, apoptosis, neural stem cells, and neuroregeneration. The efficacy of ginsenoside Rd has been studied in mice with EAE. The results were promising because the ginsenoside reduced the permeability of the blood–brain barrier, regulated the secretion of INF-gamma and IL-4 and decreased disease severity [65].

Based on the observational studies that showed that low levels of vitamin D represent a risk factor for the development of MS [66, 67], treatment with vitamin D has become increasingly attractive and has been tested in both experimental and clinical trials. Vitamin D appeared to modulate upon immune responses and inflammation, but clinical studies have not yet shown a clear benefit [68, 69].

In addition to pharmaceutical compounds, clinical and basic research studies have also highlighted that voluntary exercise can promote both neuroprotection and neuroregeneration [70, 71]. An experiment conducted in mice with EAE showed that the exercising mice (on a running wheel) presented a less severe neurological disease score, later disease onset and a significant reduction of inflammatory cell infiltration and demyelination in the ventral white matter tracts of the lumbar spinal cord [71]. Studies of patients with MS also support these observations, physical excesses determining not only improvement of muscle function and walking endurance, but also of cognitive abilities [72–75].

4. Ion channel modulation

Among the molecules that make up neurons, ion channels are especially important, because they provide them their signaling abilities. In multiple sclerosis, there were described several types of ion channels dysfunctioning:

- Ectopic distribution of calcium channels, up-regulated within the axon membrane, during the demyelinating process. Increased intracellular calcium levels activate calcium-dependent proteases (calpains) that can degrade axonal proteins, contributing to the axonal injury. Blocking the calcium channels can protect myelinated axons from axotomy-induced and anoxia-induced degeneration (see **Figure 2**) [76].

- Transcriptional channelopathy that described in cerebellar Purkinje neurons. Studies showed that Nav1.8 gene (normally inactivated in the cerebellum) is aberrantly activated in Purkinje neurons, producing the Nav1.8 protein, possibly responsible for cerebellar deficits [77].

- Ion channel dysfunctioning during remyelination—redistribution and clustering of ion channels [78–81].

In MS, excessive accumulation of Ca^{2+} ions is known to contribute to axonal degeneration in the central nervous system (CNS) through the activation of acid-sensing ion channel type 1a

(ASIC1). ASIC1 is considered a mediator of neuronal injury in stroke and CNS inflammation due to its ability to modulate Na^+ and Ca^{2+} flux. So, it could be possible to attenuate axonal loss by disrupting the ASIC1a gene or by using a nonspecific blocker of these channels, such as amiloride (a diuretic with a proven safety record) [82]. Recently, a single-arm, longitudinal trial of amiloride showed an important reduction of brain atrophy in the primary progressive form of MS. The aim of Amiloride Clinical Trial in Optic Neuritis (ACTION), an ongoing phase II clinical trial, is to demonstrate the neuroprotective effect of amiloride in acute optic neuritis (a common manifestation of MS) using a multimodal approach that combines structural and functional outcomes with clinical measures [83].
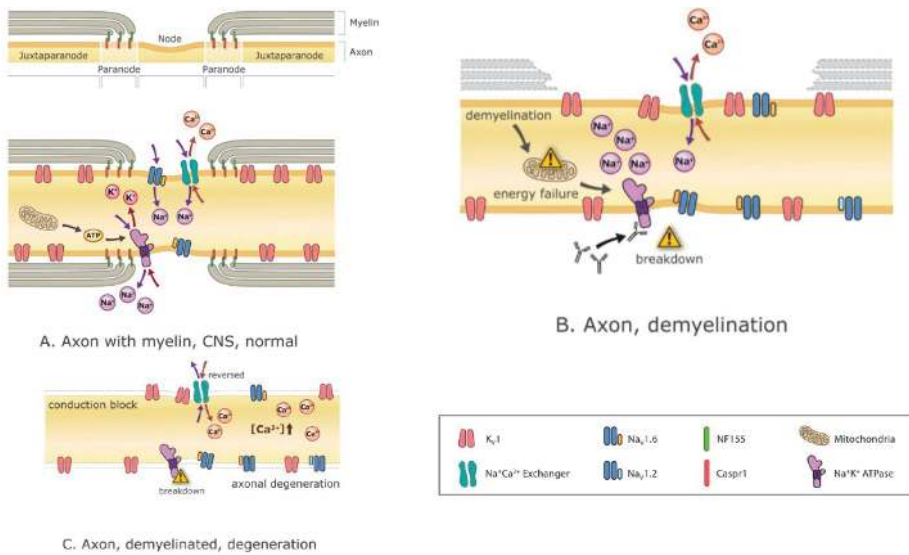

Figure 2. Mechanisms of demyelination-related neurodegeneration. Demyelination can result progressively in ionic disequilibria, energy crisis, conduction block, and eventually neurodegeneration. (A) a normal node of Ranvier with juxtaparanodal, paranodal, and nodal regions intact, depicting Na^+, K^+, and Ca^{2+} ions flowing through their respective channels with mitochondria supplying the ATP for energy-dependent Na^+K^+ ATPases that re-establish the ion gradients depleted by ion flux through channels. Numerous different ion channels are present in the axon but only a small subset is depicted here; (B) partial demyelination results in dispersal of nodal ion channels, energy insufficiency, and disequilibria of ion gradients; (C) complete demyelination can result in conduction block and axonal degeneration due to the accumulation of intracellular Ca^{2+} that results from energy crisis and disruption of ionic balances. Abbreviations: Kv1—potassium channel type 1; Nav1.6 and Nav1.2—sodium channel types 1.6 and 1.2; Na^+ Ca^+ Exchanger—Na-Ca exchange pump; Na^+K^+ ATPase—ATP (energy)-dependent Na-K exchange pump; CASPR1—contactin-associated protein 1 (interaction molecule between myelinating cell with axon); NF155—neurofascin 155 (predominant interaction molecule between myelin and axon at paranodal axo-glial junction) http://www.mdpi.com/1422-0067/16/9/21215.

4-Aminopyridine (Fampridine) is a potassium channel blocker that improves axonal conductivity in demyelinated lesions by targeting the potassium channel subtypes Kv1.1, Kv1.2, and Kv1.4 and thus correcting the leakage of potassium ions. Even if it has no impact upon disease incidence and severity, it has been already approved for improvement of fatigue, walking speed, and strength in MS patients [84].

Other potential agents that can target ion channels are lamotrigine, phenytoin, flecainide, topiramate, carbamazepine, and glibenclamide, but even if some of them have some positive results in animal studies, there is lack of clinical data regarding their efficacy in MS [85].

5. Remyelinating strategies in MS

For successful remyelination to take place, OPCs must undergo several necessary and sequential steps. This very intricate process can fail if not regulated effectively. In the first step —the activation phase—OPCs must proliferate, which involves the expression of several genes and transcription factors by either activated microglia or astrocytes within the lesion [86, 87]. Mediators such as the proteins Cdk2 and p27Kip-1, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and other factors have been demonstrated to have a proliferative effect in tissue cultures. In the second step—the migration or recruitment phase—OPCs are guided to migrate to the site of demyelination by chemotactic factors such as semaphorin receptors, neuropilins, and plexins. Semaphorin 3A impairs OPC migration to the lesion site, whereas semaphorin 3F promotes OPC migration and remyelination [88]. PDGFα is the archetypal chemotactic factor for OPCs, although it is difficult to separate its chemotactic effects from its effect on OPC proliferation. In the third step, OPCs must differentiate into remyelinating oligodendrocytes in a process driven by transcription factors such as Nkx2.2 and Olig2 [89].

Many changes in both the cytoarchitecture and microenvironment of the MS brain could prevent remyelination by endogenous OPCs. Extracellular matrix components, including fibronectin, hyaluronic acid (HA), and chondroitin sulfate proteoglycans (CSPGs), can block the differentiation of OPCs and premyelinating oligodendrocytes [90]. Components of damaged myelin, such as myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), and NOGO-A, which signal through the Nogo 1 receptor and its co-receptors p75, TROY and LINGO-1 (leucine-rich repeat- and Ig domain-containing Nogo receptor-interacting protein 1) inhibit both axonal regeneration and oligodendrocyte differentiation and remyelination [91, 92]. The differentiation phase can also be influenced by intrinsic signaling pathways (Notch signaling, Wnt signaling, and Retinoid X receptor (RXR) signaling) and extrinsic competitors (LINGO-1, semaphorin 3A, sonic hedgehog (Shh), fibroblast growth factor, insulin-like growth factor 1 (IGF-1), BDNF, chemokine CXCL 12, and bone morphogenic proteins (BMPs). The Notch signaling pathway is an important regulator of the balance between OPC proliferation and differentiation in the developing CNS as well as PNS. Notch 1 is a surface receptor expressed by both developing and mature oligodendrocytes. The ligand engaged with the Notch receptor determines whether the canonical or non-canonical signaling pathway is activated. The canonical Notch 1 signaling pathway, which is mediated through Jagged 1, prevents OPC differentiation, whereas the non-canonical signaling pathway mediated through contactin promotes differentiation [93]. The canonical Wnt-β-catenin signaling pathways negatively regulate the production and differentiation of oligodendrocytes during both developmental myelination and remyelination. Some data

suggest that the inhibition of Wnt via Axin2 promotes oligodendrocyte differentiation and remyelination [94].

Remyelination is not regulated by a single molecule or mediator but through a combination of signaling pathways that act on OPCs and oligodendrocytes as well as on other cellular players such as microglia, astrocytes, and even blood vessels. The discovery of new molecular players and of pharmacological strategies to act on them is currently a priority of the field so that new therapeutic agents that can change the natural history of MS can be developed.

Currently, from all potential remyelinating strategies for MS that stimulate OPC differentiation and enhance remyelination that include all the pathways and the signaling molecules described above [95–100], only anti-LINGO-1 antibodies have been tested in clinical trials. A phase II trial is ongoing and will provide additional information about safety, tolerability, and efficacy (NCT01864148).

The transplantation of exogenous OPCs into the CNS appears to be an attractive solution for MS, but unanswered questions render this procedure unfeasible in MS; these open questions include how to overcome the limited migration potential of transplanted OPCs, how to control the proliferation and differentiation process, and how to avoid immunosuppression treatment [101].

6. Concluding remarks from a systems biology perspective

The dynamic interactions between environmental factors and epigenetic mechanisms that involve multiple pathways and processes suggest the need for a system-based approach to understand MS physiopathology and to implement new pharmacological therapies.

Targeting neuroprotection is always ambitious, not only in MS, but in neurology in general, mostly because of a poor understanding of the complexity of interconnections between different cellular and molecular processes. In complex diseases such as MS there is a milieu of dynamical interplay between networks of genes and signaling proteins, lipids, carbohydrate molecules that can have concomitant roles in inflammation, immune systems reactivity, demyelination, neurodegeneration, neuroprotection, remyelination. For example, the network of p38 mitogen-activated protein kinase (MAPK) signaling pathway can trigger both inflammation and neuroprotection. MAPK is activated by cell stress, playing a key role in immune responses and has been intensively investigated in relation with EAE pathogenesis [102]. Taking in account this multitude of interactions, the currently trend is to inhibit/potentiate selectively a single molecular pathway, for example, acting only on p38α MAPK and not also on p38β MAPK [103].

However, over-selective interventions have an important disadvantage. Imbalances in complex systems always affect concomitant different subsystems between which there is a significant cross-talk. This leads to several pathological outcomes, for example, to inflammation, demyelination, and neurodegeneration which potentiate each other, so targeting a single pathway seems senseless. Additionally, some of these processes occur as compensatory

mechanisms and become maladaptive and trigger the emergence and expansion of vicious circles due to the alteration of modulatory mechanisms. For example, in a demyelinated axon, homeostatic plasticity that involves the redistribution of ion channels occurs, and this redistribution contributes to the failure of AP conduction and finally generates a metabolic crisis. Intercorrelation between the molecular mechanisms that underlie inflammation, apoptosis, oxidative stress, increased Ca^{2+} load, mitochondrial dysfunction, microglial activation, and blood–brain barrier dysfunction is responsible for the expansion of vicious circles that generate a nonlinear pattern of clinical evolution. From this perspective, the traditional idea of a "magic bullet" seems too simplistic to achieve sufficient neuroprotection.

An interesting explanation of these mechanisms derives from the theory of complex biological systems, which are characterized by criticality and degeneracy. Degeneracy describes the ability of structurally and functionally distinct pathways to produce the same output. This characteristic supports the existence of multifunctional components that can perform similar functions under certain conditions. A direct consequence of degeneracy is the assurance of quick compensation if one of these mechanisms fails. However, in pathological conditions, degeneracy can lead to a chronic, robust state in which a unimodal therapeutic approach that targets a single pathway will fail to ensure the sustainable irreversibility of the pathological process. According to this idea, the combination of therapies that utilize pharmacological compounds with synergic effects but different mechanisms of action or individual multimodal, pleiotropic therapies, with modulatory properties that can target as many pathways as possible offer a feasible therapeutic approach.

Last, but not least, it is very important to take in account that everyone has a different genetic polymorphism that leads to different phenotypes which can have an important influence upon the reactivity of molecular networks. This patient inter-variability may be responsible for both heterogeneity in disease progression and treatment response, leading to an open door to metabolomics [104].

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