Implications of white matter damage in amyotrophic lateral sclerosis (Review)

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Abstract. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which involves the progressive degeneration of motor neurons. ALS has long been considered a disease of the grey matter; however, pathological alterations of the white matter (WM), including axonal loss, axonal demyelination and oligodendrocyte death, have been reported in patients with ALS. The present review examined motor neuron death as the primary cause of ALS and evaluated the associated WM damage that is guided by neuronal-glial interactions. Previous studies have suggested that WM damage may occur prior to the death of motor neurons, and thus may be considered an early indicator for the diagnosis and prognosis of ALS. However, the exact molecular mechanisms underlying early-onset WM damage in ALS have yet to be elucidated. The present review explored the detailed anatomy of WM and identified several pathological mechanisms that may be implicated in WM damage in ALS. In addition, it associated the pathophysiological alterations of WM, which may contribute to motor neuron death in ALS, with similar mechanisms of WM damage that are involved in multiple sclerosis (MS). Furthermore, the early detection of WM damage in ALS, using neuroimaging techniques, may lead to earlier therapeutic intervention, using immunomodulatory treatment strategies similar to those used in relapsing-remitting MS, aimed at delaying WM damage in ALS. Early therapeutic approaches may have the potential to delay motor neuron damage and thus prolong the survival of patients with ALS. The therapeutic interventions that are currently available for ALS are only marginally effective. However, early intervention with immunomodulatory drugs may slow the progression of WM damage in the early stages of ALS, thus delaying motor neuron death and increasing the life expectancy of patients with ALS.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, involving the progressive degeneration of upper and lower motor neurons. ALS is also known as Lou Gehrig's disease, after the baseball player Lou Gehrig who was diagnosed with the disease (1). ALS can be classified as sporadic (sALS), which represents ~90% of all ALS cases, and familial (fALS), which accounts for the remaining 10% of cases (2). Sexual dimorphism has been suggested to be involved in ALS disease onset and progression (3), and the incidence and prevalence of ALS is greater in males compared with in females (4,5). Exposure to environmental toxins, such
as pesticides, has been considered a key risk factor for ALS; however, further studies are required to elucidate the implication of environmental factors in ALS development (6). ALS is the most common motor neuron disease in adults, and its onset is characterized by distal weakness in the arms or legs, indicative of lower motor neuron involvement. Muscular weakness limits the use of muscles for the execution of voluntary and involuntary movements, thus causing muscular atrophy, eventually leading to respiratory muscle failure and death (7). As the disease progresses into the later stages, patients also experience a progressive decline of cognitive function. In the later stages of ALS, the ocular muscles controlling vision are usually the last to be affected (1).

The primary focus of clinical management for patients with ALS is the symptomatic management of the disease using conventional pharmacological agents, as no cure is currently available. However, non-conventional approaches, including radiotherapy, muscle stretching and nutritional management, have also been employed to alleviate the pain, respiratory dysfunction, psychiatric/cognitive disturbances, nutritional deficits and sleep-related dysfunctions associated with ALS (8). Clinical trials for ALS started in the 1980s; however, the majority of experimental drugs proved to be ineffective with the exception of riluzole, which was reported to marginally extend the lifespan of the patients (1,9).

ALS is most commonly referred to as a motor neuron disease, due to the activation of abnormal programmed cell death signaling pathways during the pathogenesis of the disease, leading to the death and degeneration of motor neurons (10). ALS has been primarily considered a disease of the grey matter involving motor neuron degeneration; however, pathological alterations in the white matter (WM) have been reported to be more pronounced compared with those in motor neuron structures (11). In addition, WM pathophysiological processes have been detected during the early stages of ALS, prior to the appearance of clinical symptoms (12). WM is predominantly composed of myelinated and unmyelinated neuronal axons organized into specific tracts with surrounding glial cells (13). The term ‘white matter’ arises from the white color of the lipids, which are the main constituents of myelin. Myelin has a water content of ~40%; the dry mass is composed of 70-85% lipid (14) and 15-30% protein (15). Among the main proteins found in myelin are proteolipid protein (~50%), myelin basic protein (~30%) and minor proteins, including myelin oligodendrocyte glycoprotein, 2',3'-cyclic-nucleotide 3'-phosphodiesterase and myelin-associated glycoprotein (<1%) (16). The WM of the central nervous system (CNS) is susceptible to anoxia, trauma and autoimmune processes (17). WM damage can be induced by primary and secondary diseases associated with ischemia, inflammation, trauma and hypoxia (13). WM damage has been reported to contribute to the motor and neurological deficits associated with cognitive impairment in neurological conditions, including ALS (18), Alzheimer’s disease (19), Huntington’s disease (20), progressive supra-nuclear palsy (21) and multiple sclerosis (MS) (22). The severity of WM damage increases with age, leading to decreased cognitive abilities and slower conduction velocity of electrical impulses during physiological functioning (23).

The molecular mechanisms involved in WM damage vary, and include alterations in toll-like receptor-3 expression (24), absorption of Clostridium perfringens ε-toxin (25) and glutamate excitotoxicity during secondary spinal cord (SC) injury (26). Neuropathological alterations of the WM, including axonal degeneration and oligodendrocyte loss (27,28), have been reported in patients with ALS. A previous study using multi-modal magnetic resonance imaging demonstrated that cortical thinning and WM degeneration were associated with cognitive and behavioral impairments in motor neuron diseases (29), whereas significant WM differences have been identified between male and female patients with ALS (30).

The exact molecular mechanisms underlying WM damage in ALS have yet to be elucidated. The presence of clinical and pathological similarities between patients with ALS and MS suggests that a similar WM pathology may be implicated in the diseases (13,31). Typical symptoms of patients with MS include disability, incontinence, limb tremor, pain, spasms, fatigue and spasticity (32). Patients with ALS also display some of the common symptoms of MS; however, typical ALS manifestations also include severe muscle weakness, fasciculation, bulbar symptoms and severe respiratory abnormalities (33).

MS is an autoimmune disease, which is characterized by the immune destruction of the myelin sheath surrounding brain and SC neurons, leading to axonal and neuronal loss. Damaged myelin disrupts the conduction of electrical signals along neural fibers, which is essential for the maintenance of normal functions. Therefore, patients with MS suffer numerous neurological disabilities that negatively impact their quality of life. At present, no cure for MS is available, due to the inability to repair damaged myelin (34). Brain derived neurotrophic factor (BDNF) is a neurotrophin, which has demonstrated beneficial effects during remyelination processes and myelin repair; however, its actions can be hampered by the overexpression of its transcriptional repressor, methyl CpG binding protein 2 (MeCP2) (34,35). Despite the significant differences between MS and ALS, the overlapping symptoms and pathological alterations suggest that the progressive degeneration of central axons may be a critical process in the diseases.

Defects in several genes involved in axonal transport may contribute to axonal loss in MS and ALS (36). Notably, pathogenic mechanisms similar to those responsible for the development of neuroinflammation, excitotoxicity and axonal dysfunction in MS have also been identified in ALS (31). A previous study indicated that first-degree relatives of patients with MS had a greater risk of developing ALS and vice versa, thus suggesting that similar genes may predispose families to MS and ALS (37). Specifically, inflammatory T-lymphocytes, including T helper (Th) 1 and Th17, and the production of inflammatory mediators, including interleukin 6, have been reported to drive the inflammatory cascade implicated in the neurodegenerative processes present in patients with ALS and MS (38). Therefore, research has turned to other neurodegenerative diseases, including MS, in order to advance the understanding of WM damage associated with ALS and promote the development of more effective therapeutic strategies (13,39). The present review discussed the key molecules that have been implicated in the common mechanisms of WM damage in MS and ALS. In addition, current therapeutic strategies for the management of ALS were also addressed.
2. WM anatomy

The WM of the CNS contains axon bundles ensheathed by myelin, which is produced by oligodendrocytes and serves as an insulator, thus facilitating the propagation of electrical impulses along the nerve axons. There are three predominant WM tracts in the CNS, composed of projection, association and commissural tracts (40). Conversely, the CNS grey matter largely contains neuronal cell bodies, dendrites, glial cells and synapses (41). Myelin is an insulating substance, which is essential for the propagation of electrical impulses along nerve axons, and is required for the physiological functioning of the nervous system (42). The myelin sheath surrounding the nerve axons in the WM tracts serves a critical role in relaying messages from the brain to the various areas of the body; it also coordinates grey matter communication between areas of the CNS (43). Nerve axons are the predominant component of WM. They are long projections that originate from the neuronal cell body and serve to transmit information in the form of electrical impulses. In addition, nerve axons transport nutrients essential for the health and physiological function of the axons and the neuronal cell bodies (44).

In the CNS, glial cells, including oligodendrocytes and astrocytes, are critical for the maintenance of axonal integrity (45). Oligodendrocytes maintain the structural integrity and functionality of myelin, and the lipid-rich myelin sheath ensures the speed and reliability of electrical transmission (46). Astrocytes are the most prevalent type of glial cell in the CNS. WM astrocytes differ from grey matter astrocytes in morphology, development and function. WM astrocytes are mainly in charge of maintaining the neuronal-glial homeostatic equilibrium, participate in oligodendrocyte lineage differentiation, provide energy supply and nutritional support to the nervous tissue by delivering trophic factors and iron, and are involved in immune and inflammatory processes (13). Although the functional significance of WM glia has yet to be fully elucidated, previous studies have reported that oligodendrocyte and astrocyte dysfunction is implicated in the pathogenesis of neurological disorders, including MS (47), ischemic stroke (40), Huntington’s (20) and Alzheimer’s disease (48), schizophrenia (49), psychiatric disorders (50) and ALS (51).

Recently, ALS oligodendrocytes were demonstrated to contribute to motor neuron death, through a superoxide dismutase (SOD) 1-dependent mechanism (52), whereas oligodendrocyte degeneration has been reported to occur prior to disease onset. New oligodendrocytes are formed to compensate for the loss; however, they fail to mature, thus resulting in progressive demyelination (52). Furthermore, axonal demyelination has been directly associated with ALS deterioration (53). Therefore, oligodendrocyte dysfunction is considered as a major factor contributing to neuronal degeneration, with relevance to diseases including ALS (28,54). Notably, the glial pathology in ALS, including oligodendrocyte degeneration and impaired maturation, and its involvement in neurodegeneration, is analogous to the pathology displayed in MS (28).

3. Molecular mechanisms involved in the pathogenesis of WM damage in ALS

**Biological targets of ALS pathology.** Several abnormal biological processes have been suggested to be involved in the mechanisms underlying the pathogenesis of ALS (55), thus making it a very complex multi-system and multi-syndrome disorder, for which no single cause can be identified (Figs. 1 and 2). Considerable progress has been made in the investigation of the genetic aspect of ALS pathophysiology. Several genes have been demonstrated to be involved in fALS, including chromosome-9 open reading frame 72 (56), SOD1 (57), coiled-coil-helix-coiled-helix domain containing 10 (58), Matrin-3 (59), tumor necrosis factor (TNF) receptor-associated factor family member-associated nuclear factor-κB activator-binding kinase 1 (60), TAR DNA-binding protein (61), fused in sarcoma (62), optineurin (63), Valosin-containing protein (64), ubiquitin 2 (65), sequestosome 1 (66) and profilin (2,67,68).

Recently, mutations in the NIMA related kinase 1 gene were associated with ALS (69). With the development of genome sequencing techniques, the number of genes implicated in ALS pathogenesis is increasing, thus suggesting the complexity of the disease, and explaining the elusiveness of a cure. sALS and fALS are the predominant types of ALS, and appear to share some similar pathophysiological mechanisms, including oxidative stress, excitotoxicity, aggregate formation, inflammation and neurofilament disorganization (70,71).

Notably, the mechanisms underlying early-onset WM pathology in MS are similar to those responsible for early-onset WM damage prior to motor neuron death in ALS (13,72,73). Therefore, elucidation of the pathological mechanisms involved in MS-induced WM damage may provide useful insight to advance the understanding of certain aspects of ALS. Furthermore, novel therapeutic approaches that target WM pathology in the early stages of ALS, may have the potential to delay the progression of the disease and the onset of motor neuron death.

**Mitochondrial damage and oxidative stress.** Mitochondria are the primary site of ATP production; they also have a major role in the maintenance of Ca$^{2+}$ homeostasis, the production of free radicals and the regulation of intrinsic apoptotic pathways (74,75). Therefore, mitochondrial dysfunction may be involved in the initial degenerative processes of ALS (76).

Reactive oxygen species (ROS) are byproducts of aerobic metabolism. The cumulative production of ROS results in oxidative stress, which causes mitochondrial damage. ROS can induce mitochondrial DNA mutations, impair the mitochondrial respiratory chain, alter mitochondrial membrane permeability and ultimately cause cell death (77). Previous studies have suggested the time- and dose-dependent involvement of ROS, including superoxide (O$_2^·−$) and hydroxyl (OH) radicals, and hydrogen peroxide (H$_2$O$_2$), in mitochondrial damage driving motor neuron degeneration (Fig. 1) (78,79). In sALS and fALS, post-mortem and biopsy samples from the SC and motor neurons revealed abnormalities in mitochondrial structure, number and localization, which were associated with defects in respiratory chain complexes (80). Furthermore, elevated ROS production and mitochondrial dysfunction have also been observed in SC samples isolated from a rat model of sALS (81).
Figure 1. Schematic representation of the effects of mitochondrial damage and ER stress in ALS pathogenesis. Dysfunction of glial cells results in decreased levels of chaperone proteins, including HSPs and members of the PDI family. The impaired expression of chaperone proteins results in protein misfolding, which impairs ER-Golgi apparatus trafficking. The UPR signaling pathway is activated due to ER stress caused by impaired ER-Golgi trafficking, and initiates cell apoptosis. In addition, mutations in superoxide dismutase 1 cause mitochondrial damage and oxidative stress, ultimately leading to abnormalities in axonal transportation. ER, endoplasmic reticulum; ALS, amyotrophic lateral sclerosis; HSP, heat shock protein; PDI, protein disulphide isomerase; UPR, unfolded protein response; PERK, protein kinase R-like endoplasmic reticulum kinase; ATF, activating transcription factor; IRE, inositol-requiring enzyme.

Figure 2. Schematic representation of the effects of glutamate excitotoxicity, energy metabolism deficiency and axonopathy in ALS pathogenesis. Dysfunction of glial cells results in the decreased expression of glutamate transporters, including GLT-1 and GLAST, leading to glutamate excitotoxicity. Although excitotoxicity can induce axonopathy and neuronal degeneration, interventions aimed at increasing BDNF production can attenuate excitotoxicity, enhance axonal repair and regrowth and eventually ameliorate the degeneration of motor neurons. Furthermore, glial cell dysfunction results in the downregulation of MCTs, thus impairing the axonal energy supply, which leads to axon loss and motor neuron degeneration. The BDNF signaling pathway is also implicated in MCT expression; however, further studies are required to investigate the molecular mechanisms that are involved. In addition, class 3 semaphorins are involved in oligodendroglial migration, and their dysregulation is implicated in remyelination impairments and axonopathy. ALS, amyotrophic lateral sclerosis; GLT, glutamate transporter; GLAST, glutamate aspartate transporter; BDNF, brain-derived neurotrophic factor; MCT, monocarboxylate transporter; Sema, semaphorin.
Cell type-specific mitochondrial damage has been demonstrated to contribute to the pathogenesis of ALS. Mitochondrial dysfunction in astrocytes has been associated with a neurotoxic phenotype that impairs motor neuron survival (82), with a mechanism that may involve mutant (m)SOD1 aggregation. mSOD1 aggregates cause mitochondrial damage due to a decrease in the antioxidant activity of the enzyme, thus resulting in ROS accumulation. In addition, mSOD1 may potentiate the formation of OH, as suggested by the increased levels of oxidation products in mSOD1-transfected mice compared with controls (83). Furthermore, mSOD1 protein aggregates may be responsible for impairments in axonal transport, neurotrophic factor supply, endoplasmic reticulum (ER) stress and apoptosis in glial cells (84,85). mSOD1 is present in ~20% of patients with fALS and ~3% of patients with sALS (86); however, oxidative stress and mitochondrial damage are also present in non-SOD1-linked ALS cases (87).

**ER stress.** ER is an intracellular organelle that is responsible for protein quality control, as it ensures that proteins are correctly synthesized, folded, packaged and delivered in the appropriate locations. Under ER stress conditions, the unfolded protein response (UPR) signaling pathway is activated to restore cellular integrity or initiate apoptosis (88). Three ER stress sensors mediate the UPR, namely protein kinase R-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1) (89). ER stress can be triggered by the accumulation of unfolded or misfolded proteins in the ER lumen, and by other mechanisms, including the inhibition of ER-to-Golgi apparatus transport. Notably, mSOD1 has been implicated in impaired ER-to-Golgi trafficking, which represents an early cellular disturbance before the induction of ER stress, Golgi fragmentation (90), mSOD1 aggregation and cell apoptosis (Fig. 1) (91).

Chaperone proteins, including heat shock proteins (HSP) and members of the protein disulfide isomerase (PDI) family, ensure proteins are folded correctly, and may have a cytoprotective role in ALS (92). Low levels of HSP in motor neurons increase their susceptibility to stress, thus increasing their vulnerability to cell death processes. Neuronal HSP levels depend upon the neuronal and glial production of HSP (93). Notably, periventricular WM damage induced by iron accumulation in oligodendrocytes may increase ER stress and mitochondrial disruption, ultimately resulting in glial cell death (94). Therefore, WM damage may be responsible for HSP deficits and increased ER stress, which eventually lead to motor neuron apoptosis.

The UPR signaling pathway has been implicated in WM tract myelination during normal physiological conditions. The ER stress sensors PERK, ATF6 and IRE1 are activated under physiological conditions, resulting in the upregulation of downstream molecules, including PDI, 78 kDa glucose-related protein and 94 kDa glucose-related protein. These molecules have been associated with oligodendrocyte damage, reduced axon numbers and demyelination, which are associated with ALS progression (95,96). Notably, increased PDI expression has been reported in WM microglia of the SC and in astrocytes of the SC ventral horn in an ALS mouse model, thus suggesting that the UPR in WM glia may occur early in the phase of motor neuron degeneration during ALS (97).

Motor neurons in mice with fALS were revealed to be prone to ER stress and demonstrated upregulated ER stress marker expression accompanied by axonal degeneration (98). These studies suggested that the early mechanisms of WM damage in ALS may induce ER stress, which, in turn, may activate the UPR signaling pathway, ultimately resulting in motor neuron degeneration and death. Therefore, it may be hypothesized that therapeutic strategies aimed at attenuating or delaying WM damage may have potential in reducing motor neuron death and disease progression in patients with ALS, thus increasing their life expectancy.

**Glutamate excitotoxicity.** Accumulating evidence suggests that glutamate excitotoxicity may be implicated in the mechanisms of neuronal degeneration in ALS (99-101); imbalances between excitatory and inhibitory neurotransmission may contribute to the pathogenesis of the disease. Glutamate is the primary excitatory amino acid neurotransmitter in the CNS. Glutamate excitotoxicity has been associated with oligodendrocyte apoptosis and may induce WM degeneration following SC injury (26). An increase in excitatory neurotransmission, as indicated by increased levels of glutamine, has been reported in the motor cortex and WM of patients with ALS compared with healthy controls (101). Glutamine synthesis is catalyzed by the enzyme glutamine synthetase from glutamate and ammonia (102), and glutamine production is used as a marker of glutamate levels to detect glutamate-induced excitotoxicity.

Astrocyte-mediated cell-specific excitotoxicity has also been implicated in the pathogenesis of ALS. Astrocytes express two glutamate transporters (GLTs): GLT-1, also known as excitatory amino acid transporter (EAAT) 2, and glutamate aspartate transporter, also known as EAAT1, which participate in extracellular glutamate homeostasis and neuronal reuptake (Fig. 2) (103). Glutamate excitotoxicity, mediated by non-N-methyl-D-aspartate receptors, has been reported to cause axonopathy, including axonal swelling, cytoskeletal disruption and neurofilament accumulation, in the distal axonal segments of SC motor neurons (104). Studies suggested that glutamate excitotoxicity may be implicated in axonopathy, WM damage and long-term cognitive deficits in patients with ALS. Therefore, neuroprotective agents, including vasoactive intestinal peptide, may attenuate excitotoxic damage, and also increase BDNF production to promote secondary repair and axonal regrowth, thus limiting WM damage and ameliorating motor neuron degeneration (105,106).

The cell-specific effects of astrocytes have also been reported to participate in the activation of protein kinase C and mitogen-activated protein kinase (MAPK) pathways to induce neuroprotection (105,106). These results suggested that astrocyte activation may differentially facilitate or prevent motor neuron degeneration. Further studies are required to elucidate the differential functions of astrocytes in the pathology of degenerative diseases, including ALS (107) and MS (108).

**Energy metabolism deficiencies.** The human brain utilizes glucose and monocarboxylates, such as lactate, as primary energy sources. Lactate accounts for ~33% of the total energy substrates used by the brain, representing a more important fuel source for brain metabolism than glucose (109). Monocarboxylate transporters (MCTs) are responsible for...
lactate and pyruvate transport. In the brain, three MCT isoforms have been identified, namely MCT1, MCT2 and MCT4, which are implicated in lactate flux in the CNS (109). MCT1 is expressed in astrocytes and oligodendrocytes, whereas MCT4 is expressed exclusively in astrocytes. WM astrocytes and oligodendrocytes are critical for the production and maintenance of myelin (110), and the delivery of essential energy (111), thus supporting the physiological function of the CNS. Oligodendrocytes exhibit higher MCT1 expression, and increased lactate oxidation and lipid synthesis compared with astrocytes (13). Therefore, oligodendrocyte dysfunction may contribute to motor neuron degeneration and death in ALS. Notably, oligodendrocyte pathology becomes apparent prior to disease onset and persists during disease progression (45,112). A previous study suggested that oligodendroglia may support axon survival and function through a myelin-independent mechanism, whereas deficiencies in energy metabolites may underlie neurodegeneration (113). In human studies and preclinical mouse ALS models, MCT1 expression was revealed to be decreased in affected brain regions, resulting in insufficient energy supply to the axons, thus leading to axon loss and motor neuron degeneration and death (13,111,114). These findings suggested that the molecular mechanisms involved in early-onset WM damage in ALS may also contribute to motor neuron death (Fig. 2). Therefore, therapeutic strategies aimed at attenuating early-onset WM damage may have potential for the effective treatment of patients with ALS.

Unlike MTC1 or MTC4, MTC2 is highly expressed in the dendrites and axons of CNS neurons (115). Notably, a marked downregulation in the axonal expression of glucose transporter 3 (GLUT3) and MCT2 has been reported in WM samples isolated from MS lesions, and has been suggested to impede the supply of essential nutrients (116). Furthermore, Robinet and Pellerin (117) suggested that BDNF signaling may be implicated in the upregulation of MCT2 expression in brain neurons following acute exercise; BDNF may increase neuronal MCT2 expression through the translational regulation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt/mechanistic target of rapamycin/S6, p38 MAPK, and p44/p42 MAPK pathways (117).

Axonopathy. Motor neuron axonopathy has been proposed as an early initiating mechanism of ALS. Motor neuron pathology in ALS has been suggested to begin, at distal axon sites and proceeds in a retrograde manner, eventually leading to motor neuron degeneration, a hypothesis termed the ‘dying back’ mechanism (27). Axonopathy has also been demonstrated in animal models of ALS, including zebrafish (118), mice (119) and rats (120). In rats carrying the SOD1-G93A mutation, mitochondrial accumulation of mSOD1 was observed in motor neuron axons in discrete clusters located at regular intervals, instead of a homogeneous axonal distribution (120). Overexpression of mSOD1 (118), and excitotoxicity (104), have been suggested to trigger axonopathy. In addition, excitotoxic axonopathy has been associated with the aberrant colocalization of phosphorylated and dephosphorylated neurofilament proteins, which may subsequently induce axonal transport disruptions and swelling. In addition, axonopathy has been associated with abnormalities within the glial environment (121). Fast-fatigable motor neurons are highly susceptible to axonal degeneration, which is associated with deficiencies in protein and lipid supply to axons (27,122). As aforementioned, oligodendrocytes regulate axonal myelination to maintain axonal function, whereas astrocytes provide structural and trophic support for neurons. Abnormal glial-axonal interactions have been reported to be implicated in axonal swelling, neurofilament perturbations and microtubule transport defects during axonal degeneration (123).

Semaphorin proteins serve as axonal growth signaling cues and are responsible for axon guidance and neurofilament organization during nervous system development (124). Class 3 semaphorins are involved in oligodendroglial migration and remyelination. Semaphorin (Sema) 3A is a repulsive guidance cue for neuronal and glial cells, and induces the redistribution and depolymerization of actin filaments that results in growth cone collapse. In addition, Sema3A is expressed in MS lesions, where it impairs the recruitment and differentiation of oligodendrocyte precursor cells (OPCs) and inhibits remyelination (125,126). Conversely, Sema3F is an attractive guidance cue, which assists OPC recruitment and promotes axonal remyelination (126,127). The roles of Sema3A have also been investigated in ALS. In an ALS mouse model, Sema3A and its receptor neuropilin 1 were demonstrated to induce distal axonopathy (128). Furthermore, in humans, Sema3A levels in the motor cortex were significantly upregulated in patients with ALS compared with in controls. These results suggested that the increase in Sema3A expression may be implicated in axonal degeneration, and may be associated with the axonopathy and denervation that are observed in patients with ALS (129). Sema3A, and other class 3 semaphorins, are important regulators of axonal remyelination and of the immune responses that govern neuronal regeneration (Fig. 2) (130). Therefore, the inhibition of Sema3A may have potential as a novel therapeutic strategy for the treatment of patients with ALS. According to the ‘dying back’ hypothesis regarding motor neuron pathology (27), it may be necessary to focus on motor axons and nerve terminals in order to effectively delay or prevent motor neuron degradation (131).

Neuronal cell death. Several mechanisms, including oxidative stress, the aggregation of misfolded and mutant proteins, and excitotoxicity, may disrupt the homeostasis of motor neurons, ultimately causing cell death. MeCP2 is a nuclear protein with numerous biological functions, which serves a critical role in myelin damage in neurological conditions, including epilepsy (132) and MS (34). MeCP2E1 and MeCP2E2 are the two predominant isoforms of MeCP2 that exert diverse biological effects on neuronal survival. Previous studies have revealed that MeCP2E2 promotes neuronal death and apoptosis; however, these effects may be inhibited by Forkhead box protein G1 and Akt, which enhance neuronal survival (133,134).

MeCP2E1 has been reported to repress BDNF transcription, thus resulting in the failure of myelin repair mechanisms (34). BDNF serves a role in myelin repair and promotes the health of neurons, astrocytes and oligodendrocytes; therefore, BDNF deficiencies have been implicated in the pathological mechanisms of ALS (135,136). Notably, BDNF serum levels have been revealed to be significantly decreased in patients with ALS compared with in controls (137). Therefore, BDNF may have potential as a biomarker to reflect disease activity, and may
serve as a basis for the development of novel therapeutic strategies for ALS treatment (135). Neurotrophic factors, including BDNF, have been reported to exert beneficial effects in mouse models of ALS. Treatment of SOD1-G93A transgenic mice with neurotrophic factors has been reported to inhibit neuromuscular junction degeneration, enhance axon survival, delay the onset of ALS and prolong the average lifespan of the mice (138,139).

In ALS, apoptosis is the most common form of motor neuron death, and involves pro- and anti-apoptotic gene expression, caspase activation, cytochrome c release and apoptosis-inducing factor (AIF) nuclear translocation (140-142). Notably, in patients with ALS, apoptotic processes are not restricted to motor neurons, but also affect other neuronal and non-neuronal components of the CNS (143). A previous study reported increased neuronal apoptosis, accompanied by an increase in glial fibrillary acidic protein-positive astrocytes and increased microglia activation in the white and grey matter of several CNS regions (144). In addition, astrocytes, but not microglia, cortical neurons or myocytes, were suggested to have an integral role in the death of motor neurons in ALS (145).

At least three different molecular pathways have been reported to participate in programed cell death: the mitochondrial pathway, the death receptor pathway and the ER pathway (146). The present review explored only mitochondria-dependent apoptosis, as it is primarily responsible for neuronal and non-neuronal cell death in ALS. The mitochondrial apoptotic pathway is activated during the early stages of ALS, and proapoptotic signaling has been revealed to directly induce neuronal dysfunction (147). B-cell lymphoma (Bcl)-2 family members have also been reported to serve critical roles during apoptosis that lead to motor neuron death, via controlling mitochondrial permeability in ALS models. The expression and distribution of Bcl-2, Bcl-extra large, Bcl-2-associated death promoter (Bad) and Bcl-2-associated X protein (Bax) are altered in ALS mouse models, thus suggesting the presence of mitochondrial damage (143,148). In addition, inhibition of the PI3K/Akt signaling pathway can directly induce proapoptotic proteins, including Bad and Bax, and thus contribute to the degenerative and apoptotic pathways during ALS pathogenesis (149). Caspase activation and elevated cytosolic cytochrome c levels have also been observed in ALS cell lines, thus indicating that mitochondria-dependent apoptosis may contribute to cell death in ALS (150). However, the altered expression of Bcl-2 family proteins, the inhibition of PI3K/Akt signaling and the activation of caspases may not be the only pathways leading to motor neuron degeneration in ALS, as apoptosis is a complex process, and is known to be induced through numerous pathways (151).

AIF is a key regulator of caspase-independent apoptosis, and its increased expression has been associated with the progression of ALS. AIF has been revealed to co-translocate to motor neuron nuclei with cyclophilin A; following binding with cyclophilin A, AIF may induce mitochondrial membrane permeabilization and cell death in a model of ALS (152). Therefore, proapoptotic signaling may contribute to the neuronal and non-neuronal degeneration that causes WM damage and motor neuron death in ALS. Therefore, inhibition of the mitochondrial apoptotic pathway may have potential as another novel therapeutic approach to suppress myelin damage and/or preserve motor neuron viability and function in patients with fALS (147).

Microbiome. The gut microbiome can influence host biology and contribute to WM damage in CNS disorders. The microbiome-gut-brain axis is responsible for the association between the microbiome and neuroimmune and neuropsychiatric disorders (153), including MS (154,155), autism (156) and ALS (157). The microbiome-gut-brain axis refers to the interactions between the CNS, the gastrointestinal tract and the microorganisms in the gut. Several mechanisms have been suggested to explain the influence of the gut microbiome on brain health (158): Impaired intestinal barrier function has been suggested to promote the passage of toxins from the intestinal lumen into the blood circulation and the brain. A previous study demonstrated that *Clostridium perfringens* v-toxin may be responsible for WM damage in the CNS of mice. v-toxin secreted into the gut was revealed to bypass the blood-brain barrier and cause mature oligodendrocyte death, demyelination and WM injury; these effects were dependent on the expression of myelin and lymphocyte protein proteolipid (25). Notably, v-toxin has been demonstrated to exert selective toxic effects on oligodendrocytes but not astrocytes, microglia or neurons in primary cultures (25). Furthermore, dysbiosis of the gut microbiota has been reported in patients with MS compared with in healthy controls (159,160), whereas gut-derived neurotoxins have been proposed as a cause of ALS (161,162). In an ALS mouse model (SOD1-G93A), impaired gut integrity and a shift in the profile of the gut microbiome have been observed at the early stages of the disease, and have been reported there to be associated with increased disease severity (163). These findings suggested a potential role for the microbiome in the progression of ALS. However, the precise alterations in the gut microbiome during ALS pathogenesis have yet to be elucidated. Numerous factors, including hygiene, antibiotic usage, microbiota composition, probiotics and diet, have been proposed to influence the link between the gut microbiome and CNS (153). Understanding the relationship between the gut microbiome and neuroimmunology may aid the development of novel preventative and therapeutic strategies for the treatment of CNS disorders, including ALS.

4. Therapeutic strategies aimed to attenuate or delay WM damage and disease progression

Riluzole. Similar mechanisms have been proposed concerning the pathogenesis of WM damage and subsequent disease progression for ALS and MS; however, no cure exists for these chronic diseases. Currently available therapeutic interventions mainly focus on delaying the onset of the disease, slowing its progression and improving the survival rates.

Riluzole is the only drug approved by the US Food and Drug Administration for the treatment of patients with ALS, and it has been clinically available since 1995. However, treatment with riluzole can only marginally improve the neurological symptoms of the patients and prolong their survival by 3-4 months (164), whereas a previous epidemiological study reported that riluzole exerted a beneficial effect only during the first 6 months of therapy, with an apparent reversal of its beneficial effects after the 6-month time point (165).
The molecular mechanisms underlying the neuroprotective effects of riluzole in ALS have yet to be elucidated. It has been suggested that riluzole may exert its beneficial effects via preventing motor neuron excitotoxicity, through the blockade of voltage-dependent ion channels (166-168). However, riluzole has also been reported to act on astrocytes in the WM to induce neural growth factor production and improve the neuronal survival rate (168,169). In addition, it has been demonstrated to stimulate BDNF release (170,171). A previous study from our group suggested the importance of BDNF during myelin repair, as increased levels of BDNF were revealed to facilitate myelin repair and offer neuroprotection in the CNS (33). Therefore, the enhancing effects of riluzole on BDNF production may also contribute to its neuroprotective effects in patients with ALS.

Riluzole has been demonstrated to reduce inflammation, demyelination and axonal damage, and attenuate the clinical severity of the experimental autoimmune encephalomyelitis (EAE) model of MS, thus suggesting that riluzole may also be beneficial for the treatment of MS (172). A phase II clinical trial is currently in progress for the use of riluzole in patients with MS (173); however, a previous phase II trial in patients with early MS revealed that riluzole was not able to prevent the progression of brain atrophy (174). Furthermore, the acute and chronic treatment of ALS mice with riluzole exerted opposite effects on the production of trophic factors in the CNS, including glial cell-derived neurotrophic factor, BDNF, cardiotrophin-1 (CT-1) and nerve growth factor. Acute treatment with riluzole was revealed to induce trophic factor production in the SC, sciatic nerve and brain, whereas chronic treatment exerted inhibitory effects (169). In the study by Dennybs et al (169), riluzole significantly increased CT-1 levels in the SC following 15 days of continuous treatment, which returned to baseline following 30 days of treatment. In addition, riluzole increased brain BDNF levels following 6 and 15 days of treatment, which were significantly decreased following 30 days of treatment.

The levels of released BDNF appear to be a critical factor in ALS pathology, since BDNF has been reported to serve an essential role in the development of pathologic pain (175). Increased BDNF levels have been suggested to induce the development of chronic pain, whereas BDNF deficits may result in the failure of myelin repair mechanisms (34,176). These findings suggested that a delicate equilibrium in the endogenous BDNF levels may be required for the maintenance of myelin repair without the induction of nociception. Therefore, therapeutic schemes that favor the acute effects of riluzole administration, and the close monitoring of BDNF levels may have the potential to improve the therapeutic outcomes of treatment with riluzole.

Other drugs on the market or in clinical trials. As the efficacy of riluzole is only marginal, and its administration can increase survival by only a few months, clinicians suggest that treatment with riluzole should be started at the early stages of the disease in order to maximize its benefits. Novel agents with higher efficacy are currently under investigation, and various administration routes are being evaluated in order to improve the efficacy and minimize the adverse effects of the treatments (177).

Novel experimental drugs are currently being evaluated in preclinical animal models and in human clinical trials (178). Pramipexole (PPX), is a D2/D3-prefering dopamine receptor agonist, which has been demonstrated to exert beneficial effects in the EAE model of MS (179); PPX blocked neuroinflammatory responses, demyelination and astrogial activation in the SC, and it inhibited the production of proinflammatory cytokines and ROS (179). In addition, dexpramipexole (RPPX), which is the R (+) enantiomer of PPX, has also demonstrated neuroprotective effects, via acting directly on mitochondria to stabilize mitochondrial ionic conductance and reduce free radical production, thus inhibiting cell death (180-182). Early phase clinical trials in patients with ALS suggested that RPPX has a promising safety and tolerability profile, and a phase III clinical trial is currently underway to investigate its efficacy in patients with ALS (183).

Pioglitazone is a peroxisome proliferator-activated receptor-γ agonist, which has been demonstrated to exert anti-inflammatory and neuroprotective actions. It has been suggested as a potential therapeutic agent for the treatment of MS, due to its ability to reduce TNF-α-induced myelin damage and mitochondrial dysfunction (184). In a phase I clinical trial in patients with relapsing remitting MS, treatment with pioglitazone was reported to reduce lesion development in WM, via inhibiting demyelination and axonal degeneration (185). However, pioglitazone did not exert beneficial effects on the survival of patients with ALS, as revealed by a phase II clinical trial evaluating it as an add-on therapy in combination with riluzole (186). However, riluzole was revealed to exert neurotoxic effects at concentrations between 3 and 30 μM, which may antagonize the neuroprotective effects of several compounds being evaluated in clinical trials, including resveratrol, memantine, minocycline and lithium (187). Therefore, further studies are required, using a group of patients without riluzole treatment to evaluate the neuroprotective potential of novel agents in ALS (187).

Flavonoids are bioactive compounds that are derived from fruit and vegetables. Epigallocatechin-3-gallate is a flavonoid that has been demonstrated to reduce neuroinflammation, and limit demyelination and axonal damage in the EAE model of MS (188) and the SOD1-G93A mouse model of ALS (189). The neuroprotective effects of flavonoids suggest that they may have potential as alternative therapeutic agents for the treatment of neurodegenerative diseases, including MS and ALS (190).

Stem cell transplantation has also been recognized as a potential therapeutic strategy for the treatment of patients with ALS and MS (191). A previous study demonstrated that a neural stem cell (NSC) population isolated from human induced pluripotent stem cells improved the neuromuscular function and increased the life span of ALS mice, following intrathecal or intravenous administration. The results revealed that the transplanted NSCs migrated and engrafted into the CNS, where they improved the production of neurotrophic factors and reduced micro- and macrogliosis (192). Furthermore, a human study demonstrated that transplantation of autologous stem cells into patients with ALS delayed disease progression and increased survival (193). In addition, neural precursor cell transplantation has been reported to
enhance remyelination in an EAE mouse MS model of extensive demyelination (194). Genetically engineered bone marrow stem cells have also been used to deliver BDNF in EAE mice, and resulted in the significant delay of EAE onset, which was accompanied by a reduction in demyelination and overall clinical severity (195).

ALS is a multi-syndrome disease, which is characterized by extensive genetic and phenotypic variability. Therefore, the discovery of a single agent that can be used for the treatment of all ALS patients is unlikely. Although MS and ALS differ in many aspects, they share a number of common pathogenic features, including inflammation, oxidative stress, mitochondrial dysfunction and WM damage (196). For this reason, early diagnosis, and early interventional therapies that target certain molecular and genetic pathways are urgently required for the treatment of patients with ALS and MS.

5. Conclusion

ALS is a complex multi-system and multi-syndrome disease that affects neuronal and non-neuronal populations, and is characterized by the progressive degeneration of motor neurons. Several pathological mechanisms are involved in WM damage in ALS, including mitochondrial dysfunction, oxidative stress, neuronal apoptosis, ER stress, glutamate excitotoxicity, energy metabolism defects and axonopathy, which bear a strong similarity to other WM disorders, such as MS (Figs. 1 and 2). Therefore, immunomodulatory agents that are currently available for the treatment of MS may have potential as early treatment options for patients with ALS characterized by early-onset WM damage.

The current review presented a comprehensive evaluation of ALS, discussing motor neuron death as the principal cause of the disease, and examining the impact of early-onset WM damage, which is a common pathology in ALS and MS, as confirmed by neuroimaging techniques (12,197-199). The immune system has been identified as a key regulator of pathological neuronal-glial interactions. However, the exact molecular mechanisms surrounding WM damage in ALS have yet to be elucidated. Although MS and ALS are distinct neurodegenerative CNS diseases, they share common pathogenic features. Therefore, understanding the molecular mechanisms that underlie WM damage in MS may aid the development of improved therapeutic strategies that address the early-onset WM damage occurring in ALS. The identification of potentially important molecular targets, including MeCP2E1, MeCP2E2, BDNF (34) and semaphorin (200,201) in MS may help advance our understanding of the molecular mechanisms underlying the pathogenesis of ALS. Current agents used for the treatment of ALS, including riluzole, and experimental drugs currently in clinical trials do not appear to affect the WM damage that is associated with the disease. Further studies are required to elucidate the roles of WM damage and neuroglial pathology in the development and progression of ALS.

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References


112. Pierre K and Pellerin L: Monocarboxylate transporters in the (4)

113. King AE, Dickson TC, Blizzard CA, (4)


115. Pie S, Tepavcevic V, (4)


136. NGO, 2012 (Epub ahead of print).


