ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the loss of upper and lower motor neurons in the motor cortex, brain stem, and the anterior horn of the spinal cord. The majority of ALS cases are classified as sporadic (sALS). There is a growing concern regarding the increased incidence in the number of sporadic ALS cases across the world, projected to increase by almost 70% in the next two decades. The etiology of sporadic ALS is currently unknown; however, epidemiological studies point to possible exposure of environmental triggers, including trauma and infections as risk factors for the development of motor neuron pathology. On a pathological basis, protein misfolding with the accumulation of cytoplasmic inclusions of TDP-43 are regarded as the hallmark feature of ALS pathogenesis. The cellular mechanisms that lead to protein aggregation are not completely understood, but appear to involve defects in autophagy, an intracellular autodigestive process that degrades misfolded proteins like TDP-43. This review will be split into two portions: (1) discuss the evidence regarding how various environmental risk factors, such as infections agents and physical trauma, can lead to neuropathological changes by disrupting autophagy in ALS; (2) discuss potential treatment options in the management of each environmental factor previously discussed.

Keywords: ALS; Infectious; Autophagy; TBI; Neurodegeneration
INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is regarded as an invariably fatal neurodegenerative disease characterized by a progressive loss of motor neurons in the spinal cord, brainstem, and corticospinal tract that commonly presents with progressive asymmetric distal weakness and/or bulbar dysfunction. Respiratory failure typically limits survival to 2–5 years after disease onset, although the clinical presentation and course of ALS can be quite heterogeneous with a high degree of variability in the age at onset, the site of onset, and the rate of disease progression among different patients.1–4 In fact, clinical heterogeneity exists even between those with the same genetic familial form.5

In ALS, one of the primary misfolded proteins is TDP-43, a 414 amino acid-long nuclear RNA binding protein linked to familial and sporadic cases.6 TDP-43 mutations at the C-terminal increase its propensity to aggregate, and it has been shown to disinhibit viral expression, which in turn can provoke a heightened immune response characterized by pro-inflammatory cytokines such as transforming growth factor-beta (TGF-β).7,8 The connection between TDP-43 and RNA pathology from viral or enteropathogens was initially made based upon the discovery that TDP-43 inhibits HIV-1 gene expression by blocking the assembly of its transcription complexes.9 A further clue that ALS can be precipitated by RNA viruses is based upon the fact that several pathogens exhibit tropism for anterior horn cells, including enteroviruses, coxsackievirus, echovirus, polio, HIV, HTLV-1, and HERV-K.10 As mentioned, retrovirus activity and other microbial pathogens can elicit the production of pro-inflammatory cytokines such as TGF-β, which in turn induces membrane fibrosis leading to vascular remodeling, impaired perivascular cerebrospinal fluid (CSF) distribution, reduced compliance of lymphatic vessels, and defects in autophagy.11 Autophagy is a multistep pathway for intracellular and extracellular processes that are essential to degrade pathological protein aggregates and removal through the lymphatic system. The lymphatic system is a glial cell-mediated lymphatic system of the brain that can be disrupted by several environmental factors. From an intracellular perspective, functional lysosomes digest misfolded proteins through pH-dependent proteolytic enzymes. However, if lysosomes are downregulated, extracellular clearance is required to remove macromolecules from the interstitial fluid via the CSF and lymphatic system. Environmental factors, notably sleep disturbances, infections, and repetitive head injuries, all have been proposed as risk factors for the development of ALS. This review will discuss the pathophysiology of ALS and propose addressing the neurogenerative disease utilizing a systems biology approach. A systematic approach to ALS offers a new paradigm that addresses the interdependence of the central nervous system, gastrointestinal system, and the immune system as evidence suggests that a multimodal approach rather than a single intervention may have greater effectiveness.

PART 1

TDP-43, RHO KINASES, AND INFECTIONS

TDP-43 a nuclear RNA binding protein, is intimately involved in the regulation of autophagy, an essential process that allows cells to maintain proteostasis by recognizing and degrading pathological proteins, whereas mutations in TDP-43 result in protein aggregation. One of the pathways involved in autophagy is mediated through Rho kinase signaling. Excessive Rho activation provokes neurite retraction and neuronal apoptosis, which may be linked to the pathogenesis of ALS.12,13 Rho kinases have been proposed to be intimately involved in ALS as in vivo studies utilizing pathway enrichment analysis have demonstrated significant enrichment of Rho GTPases in late-stage ALS.14 The connection between TDP-43 and Rho activity is supported by studies where the cellular knockdown of normal TDP-43 function induces cell death through the disruption of the Rho family of GTPases, resulting in reduced autophagy and the misfolding/aggregation of RNA proteins.15
The Rho family of GTPases act as master transcription termination factors and have the ability to regulate neuronal morphology and survival by providing stability for microtubules in motor neurons.\textsuperscript{16,17} Rho proteins are the targets of various bacterial enteropathogens, including toxicogenic \textit{Clostridium} species and the cytotoxic necrotizing factors from \textit{Escherichia coli}, which may exert their toxicity through inhibiting autophagy by altering GTP-binding proteins.\textsuperscript{18,19} It has been demonstrated that inhibition of the Rho GTPase family of cytoskeletal regulators causes a reduction in viral infection.\textsuperscript{20} Several RNA viruses, as well as enteropathogens, induce cytoskeletal abnormalities that target motor neurons through the activation of Rho-GTPases. In HIV infection, for example, the virus causes activation of the Rho GTPase family of cytoskeletal regulators within the host CD4\textsuperscript{+} T cells, leading to optimal infectivity.\textsuperscript{20} Furthermore, viral pathogens like HIV can spread through the enhancement of Rho-dependent migration of monocytes and macrophages.\textsuperscript{21}

Additionally, the cytotoxicity of fungal species, including \textit{Aspergillus} and \textit{Fusarium} species, involves disrupting the Rho GTPase signaling cascade through the release of gliotoxins that alter Rho signaling to favor their infectivity.\textsuperscript{22,23} \textit{Aspergillus} infection leads to the formation of actin aggregates through disruption of RNA signaling, which in turn inhibits the assembly of v-ATPase on lysosomal membranes.\textsuperscript{24} Exposure of neurons to fungal neurotoxins elicits a significant increase in abnormal TDP-43 translocation from the nucleus to the cytoplasm, which furthers the supportive link between infections and ALS pathogenesis.\textsuperscript{25} Evidence for the potential connection between fungal exposure and motor neuron disease is supported as fungal antigens and DNA have been detected in CSF and brain tissue from ALS patients using PCR analysis.\textsuperscript{26} In summary, disruption of TDP-43 pathways involving Rho kinases may be secondary to infection-mediated toxins that disrupt microtubules.

**INFECTION PATHOGENESIS**

It is well established, for instance, that motor neuron pathology can be precipitated by enteropathogenic viruses that exhibit tropism for anterior horn cells, such as poliovirus. A growing list of RNA viruses, bacterial, and fungal species are capable of remaining dormant in the nervous system by eluding host immune defense mechanisms by inhibiting lysosomal-mediated protein degradation. Lysosomes promote autophagy and are an essential element of the innate and adaptive immune system for antigen processing.\textsuperscript{27,28} Multiple intracellular organisms survive within their hosts based upon their ability to impair lysosomal-based enzymes responsible for degrading pathological proteins formed by infectious particles.

It has been proposed that many intracellular pathogens resist sterile eradication based upon their ability to inhibit lysosomal vacuolar ATPase.\textsuperscript{29,30} Thus, the connection between ALS and various microbial toxins is linked to the disruption of host vacuolar-type ATPase (v-ATPase), leading to reduced lysosomal-mediated autophagy.\textsuperscript{31} Indeed, loss of v-ATPase-mediated lysosomal acidification has been proposed as a primary mechanism in ALS pathogenesis.\textsuperscript{32} Further support for an infectious etiology in ALS is based upon the unexplained findings of defects in iron homeostasis in patients. In several studies examined by meta-analysis, serum iron levels were shown to be lower and ferritin levels are higher in ALS patients.\textsuperscript{33} Iron deficiency may result as a consequence of enteropathogens that divert dietary iron from their host, whereby microbial infections hijack iron delivery from hosts to promote their survival.\textsuperscript{34,35} \textit{E. coli}, for instance, acquires enhanced virulence through overexpression of several iron acquisition systems leading to host iron deficiency.\textsuperscript{36}

**THE ROLE OF IRON IN PATHOGENESIS**

Lysosomes, as the intracellular site of autophagy, are involved in both innate and adaptive immune functions, including foreign material recognition (bacterial, parasitic, and viral) and antigen processing.\textsuperscript{28} Multiple intracellular organisms survive within the lysosome based upon parasitic-mediated deacidification mechanisms resulting in defective xenophagy – a form of autophagy where host cells ingest foreign antigens – by interrupting lysosomal vacuolar ATPase and its ability to promote lysosomal acidification.\textsuperscript{29,30}
The chronicity of infectious diseases occurs by their ability to hijack RNA metabolism and thereby interrupt vacuolar inhibit lysosomal vacuolar ATPase and its ability to promote lysosomal acidification. In ALS, it is feasible that host Rho proteins are the targets of various bacterial and viral toxins that collude to cause cell death by inhibiting the assembly of host v-ATPase, disrupting lysosomal activity and autophagy. Indeed, loss of v-ATPase-mediated lysosomal acidification has been proposed as a primary mechanism in ALS pathogenesis.

Lysosomes are reservoirs of reactive ferrous iron that possess a system for cation transport through endosomal Fe(2+) transporter that coordinates iron metabolism, where it functions to transfer the endosomal free Fe(2+) into the cytoplasm. Unbiased genetic screens discovered that lysosomal acidity was maintained via iron homeostasis. Iron deficiency in ALS patients can lead to lysosomal v-ATPase inhibition and defective xenophagy, and the maintenance of a highly acidic pH is dependent upon iron homeostasis, which is essential for regulating many functions of lysosomes, including the activity of the v-ATPase to maintain autophagy. It is worth noting that defects in v-ATPase activity and alkalinized lysosomes have been reported in ALS.

Iron deficiency results in the up-regulation of divalent metal transporter 1 (DMT1), an iron-transport protein that contributes to antimicrobial function. The gene that encodes DMT1 iron transport has been reported to result in a faster rate of progression than in ALS patients who do not harbor the same gene defect. It is interesting to note that DMT1 normally acts to confer resistance against bacterial infection by regulating proton/metal-ion cotransport in lysosomes. However, it was recently demonstrated that hepatitis C and other viral pathogens may inhibit the expression of DMT1 to redirect iron towards viral transcription. Thus, the virulence of chronic intracellular infections is facilitated by interrupting host post-transcriptional regulation of iron transport through DMT1. Lysosomal dysregulation through alkylation and dysfunctional DMT1 are major pathogenic factors that contribute to the accumulation of protein aggregates and may represent the root cause of ALS pathogenesis.

INFECTION-MEDIATED ALTERED IMMUNE HOMEOSTASIS

Loss of immune homeostasis and/or early deregulation of immune responses may represent one of the key initial steps in disease pathogenesis in ALS, leading to either excessive inflammation and/or an inefficient immune response. For example, ALS progression has recently been intricately linked to patient T cell profiles. T regulatory cells (Tregs) that balance the activation/inhibition of the immune system’s response to infection negatively correlate to disease severity, that is, low quantity are predictive for rapid disease progression and short survival. Further, T cell subtypes Th1 and Th17 presented a shift towards cytokine production, including interferon-gamma, whereas anti-inflammatory Th2 and T regulatory cells were decreased. Pro-inflammatory serum cytokines include interleukin (IL)-1β, and IL-6. In addition to declining Treg activity, TGF-β1 – an anti-inflammatory cytokine – has been reported as a potential marker of disease ALS severity as there is a strong negative correlation between the cytokine and ALS function. Elevations of TGF-β levels are often found in association with chronic infections as TGF-β is produced by the infected intestinal epithelium, including Helicobacter pylori, and hepatitis B and C virus. Despite its typically known anti-inflammatory activity, it is interesting to note that elevated levels of TGF-β can adversely affect the glymphatic system by inducing fibrosis in the perivascular network of blood vessels, leading to impaired perivascular CSF distribution and reduced compliance of glymphatic vessels. In clinical studies of ALS, evidence for the involvement of TGF-β in ALS pathogenesis includes findings of enhanced fibrosis and TGF-β1 immunoreactivity in ALS patients greater than twofold over controls. Evidence of systemic fibrosis has included myocardial imaging demonstrating increased magnetic resonance imaging (MRI) T1 enhancement in ~80% of ALS patients compared to ~25% of controls with a trend of myocardial fibrosis observed in ~25% of the patients and ~10% of controls.

VASCULAR DYSFUNCTION

In a similar manner as chronic infections, bacterial infections, including periodontal bacteria, gut
Microbiota, *Helicobacter pylori*, and *Chlamydia* are linked to the pathogenesis of atherosclerosis through immunological cross-reactions. Thus, ALS may involve infection-mediated changes in the vascular system as well. Evidence for vascular dysfunction in ALS has been supported by a number of studies, including peripheral arterial tonometry, which revealed significant declines of peripheral arterial endothelial functions in ALS, a marker of vascular dysfunction. Microvascular disturbances involving the degeneration of endothelial cells surrounding the blood–brain barrier and in the ventral horn of patients have also been reported, demonstrating the role of vascular degeneration in ALS pathogenesis.

TDP-43 has been shown to result in reduced blood circulation and alterations in vascular function. The protein deposits have been found both as cytoplasmic inclusions and in carotid and cerebrovascular plaques. This strongly suggests that ALS not only involves TDP-43-mediated alterations in motor neurons but involves the vascular system as well. Furthermore, TDP-43(+) inclusions reduce blood circulation and may cause aberrant vasculogenesis in association with motor neuron pathology.

**TRAUMA-MEDIATED GLYMPHATIC DYSFUNCTION**

As mentioned previously, head injuries pose a risk factor for sporadic ALS. The connection between traumatic brain and spine injuries and neurodegeneration is supported by strong epidemiological evidence of repetitive head injury as an independent risk factor for the development of sporadic ALS. Moreover, consortium studies found that two or more head injuries are associated with a greater than threefold increased risk of ALS. Head and neck trauma may lead to disruption of the blood–brain barrier in tandem with ischemic changes and vascular hypoperfusion of motor neurons. In autopsied samples of the cervical spinal cord from ALS patients, perivascular hemoglobin deposits with the accumulation of fibrin and thrombin were found in the majority of patients. Furthermore, TDP-43 levels are differentially affected by the number and magnitude of blast exposures, increasing following a greater number of exposures at various intensities. At the spinal cord level, ischemic changes may be precipitated by dural displacement and epidural venous swelling in the spinal cord. For instance, in Hirayama disease, a disorder in the differential of ALS, results in ischemic myelopathy due to displacement of the dura, and perivascular compression is best visualized by dynamic flexion MRI studies. Support for structural impediments in motor neuron disease includes recent MRI findings that have confirmed cervical spine abnormalities in patients with ALS and reduced CSF flow.

The glymphatic system refers to a network of venous perivascular spaces that directs interstitial fluid into the CSF. The glymphatic system extends longitudinally down the entire length of the spinal canal through perivertebral veins that can become compressed due to neck trauma. Support for glymphatic failure in neurodegeneration has been demonstrated in Alzheimer disease (AD), where amyloid deposits were found in high concentrations in the cervical and axillary lymph nodes, which are part of the glymphatic system. Further support for glymphatic failure in neurodegenerative disease was the recent finding that efflux of CSF containing amyloid-β and phosphor-tau has been reduced in patients with AD, compared to age-matched controls. Parkinson disease has also been associated with a sustained reduction of CSF-glymphatic fluid transport.

Comparison of ALS and control groups revealed a reduction in CSF flow magnitude and increased flow propagation velocities in the ALS cohort, with the ALS cohort displaying nearly zero CSF flow along the entire spinal canal, supporting the notion that glymphatic dysfunction is directly linked to ALS pathogenesis. Recent findings suggest that glymphatic dysfunction may be the root cause of autophagic defects after traumatic head injuries, resulting in neurodegeneration due to insufficient venous drainage in the CNS.

Trauma can also induce damages to the autonomic nervous system. In ALS, degeneration of the central autonomic network, including the hypothalamus and the vagal nerve, is disrupted as evidenced by ultrasound-based diagnostics. Impaired cardiovascular function can lead to an unexpected death in patients as autonomic dysfunction is often found in patients with neurodegenerative diseases. In summary, in a
similar manner as intracellular infections can precipitate neurodegenerative changes based upon defects in intracellular autophagy, traumatic events involving the head and neck can reduce autophagy through biophysical disruption of the lymphatic system.

PART 2

ADDRESSING INFECTION AND MITOCHONDRIAL DYSFUNCTION

As mentioned above, there is both circumstantial and direct evidence of a potential association with chronic intracellular pathogens. While the theory of ALS as an infectious disease is still in its infancy, RNA-based viral, bacterial, and even fungal pathogens have been shown to disrupt microtubules leading to motor neuron pathology. Recent findings in models of ALS have demonstrated putative benefits in inhibiting TDP-43 protein aggregates.74

Doxycycline (a tetracyclic antibiotic) and its derivatives have been shown to have neuroprotective effects by inhibiting amyloid aggregation and also inhibiting aggregation of α-synuclein, the pathological protein in Parkinson disease.74 In ALS mouse models, doxycycline acts to suppress the expression of human TDP-43 and inhibits motor neuron loss. The administration of doxycycline showed a significant increase in muscle innervation, the rescue of motor impairments, and a dramatic extension of lifespan, even after neurodegeneration and motor dysfunction.75 Interestingly, doxycycline also decreases RhoA-GTPase activity, which may be part of its neuroprotective mechanism.76 Sub-antibiotic doses of doxycycline also appear to be high enough to interfere with the production of α-synuclein toxic species, the pathological aggregate in Parkinson disease pathogenesis.77 However, multiple bacterial organisms have developed different mechanisms of resistance mediated by drug efflux mechanisms involving the TetA protein (tetracycline activating protein), which have been identified in the human gut metagenome that confers resistance to tetracycline.78,79

Resistance to doxycycline may potentially be overcome by natural monoterpenoids such as β-thujaplicin (hinokitiol). A comprehensive study found that the natural plant alkaloid overcame TetA tetracycline bacterial resistance to doxycycline.80 On its own, β-thujaplicin possesses high antimicrobial activity against various oral, nasal, and nasopharyngeal pathogens, including Streptococcus mutans, Porphyromonas gingivalis, and prevents biofilm formation in both antifungal-susceptible and antifungal-resistant strains of Candida species.81,82 β-Thujaplicin has also been shown to protect against prion-induced neurotoxicity by inducing autophagy via the activation of AMPK, which in turn increases the formation of functional autolysosomes to promote autophagy.83,84 Furthermore, β-thujaplicin binds to both ferrous and ferric iron with high affinity and maintains iron homeostasis, which can restore iron transport into, within, and/or out of cells.85

Nonpharmacologically, taurine – a semi-essential amino acid – induces the production of CD4+CD25+FoxP3+ Treg cells, which may restore the population of Treg cells and normalize inflammatory responses.86 Taurine also has demonstrated an anti-fibrotic effect in several animal models where experimentally induced elevations of TGF-β1 were significantly suppressed by taurine and that effectively reduced the abnormal deposition of collagen.87 In ALS, the immunoreactivity of the taurine transporter in spinal cord motor neurons of ALS transgenic mice and in spinal motor neurons of patients with ALS has been found to be increased.88 Taurine also has inherent antimicrobial effects in addition to its anti-inflammatory effects. Neuroinflammation is a host response to infections where neutrophils are recruited and undergo an oxidative burst to diminish microbial burden. Taurine reacts with hypochlorous acid (HOCl), an antibacterial pro-oxidant produced by myeloperoxidase, resulting in the generation of taurine chloramine (TauCl), a weak oxidant with mild cytotoxicity.89 Intriguingly, N-acetylhomotaurine (a pharmaceutical analog of taurine approved by the FDA for alcohol cessation) was shown to inhibit the accumulation of TDP-43 protein in the cytoplasm when combined with baclofen, a muscle-relaxing agent.90 This effect may be attributed in part to homotaurine’s ability to indirectly inhibit N-methyl-D-aspartic acid receptors, a primary pathological target of Riluzole, a drug approved by the FDA for ALS.91

Riboflavin is a water-soluble B vitamin that is involved in a diffuse array of metabolic processes.
involving the biosynthesis of coenzyme A, coQ10, heme, and folic acids to support methylation pathways.\textsuperscript{92} Riboflavin, perhaps by supporting mitochondrial activity, plays a role in elevating heat shock proteins as an inherent defense against microbial challenges by acting as an agonist of MHC-related protein-1, which in turn facilitates mucosal-associated invariant T cells.\textsuperscript{93} Motor neurons are selectively impaired during riboflavin deficiency states by which hypomagnesemia along with low magnesium may impede ATP production. In case studies, riboflavin at 150 mg/day improved muscle strength and reduced plasma lactate and alanine concentrations in a patient with mitochondrial complex I deficiency.\textsuperscript{94,95} To illustrate its potential, autosomal recessive mutations in the riboflavin transporter genes cause a condition referred to as Brown–Vialetto–Van Laere (BVVL), a rare hereditary form of ALS. Reduced autophagic flux in motor neurons may be partially rescued by riboflavin supplementation.\textsuperscript{96} A case report of juvenile ALS – phenotype secondary to a genetic defect in riboflavin transporter deficiency – was dramatically improved after high-dose riboflavin replacement therapy.\textsuperscript{97} It is interesting to note that riboflavin has been shown to reduce the virulence of \textit{E. coli} and \textit{S. aureus}, and the production of pro-inflammatory cytokines induced by lipopolysaccharide.\textsuperscript{93,98} Analogues of the riboflavin biosynthesis pathway, such as roseoflavin, have been shown to exert bactericidal effects by targeting and downregulating the ribolavin riboswitch in bacteria.\textsuperscript{99}

Carnitine also plays an important role in energy metabolism and has been shown to improve inspiratory capacity.\textsuperscript{100–102} A case report found that L-carnitine and riboflavin improved clinical symptoms and normalized complex I activity in a 6-year-old with myopathy (associated with complex I deficiency) and pure motor neuropathy.\textsuperscript{103} After supplementation, the patient improved, and by 18 months only slight weakness in the peroneal muscles was detected on clinical exam. While the combination of magnesium, taurine, riboflavin, and carnitine have not been previously conceived as yet for a clinical trial for ALS, its purported mechanisms of action seem worth considering to assess in patients with evidence of mitochondrial dysfunction, while also addressing the infection basis of disease pathology.

**ADDRESSING ALS AS A VASCULAR DISEASE**

RhoA/Rho-kinase pathways have been demonstrated to play a role in the pathogenesis of vasospasm, glaucoma, arteriosclerosis, ischemic injury, and hypertension.\textsuperscript{104} Fasudil, a Rho kinase inhibitor, relaxes smooth muscle and is approved for the treatment of cerebral vasospasms in Japan.\textsuperscript{105–108} Rho-kinase inhibitors are now approved for use in glaucoma by lowering intraocular pressure (IOP) and enhancing aqueous humor outflow.\textsuperscript{109,110} There is a potential association between glaucoma and ALS pathology due to common mutations in optineurin, which plays an essential role as an autophagy inducer.\textsuperscript{111–113} These findings led to an ALS study whereby fasudil showed improvement in respiratory function in a small cohort of patients that was followed by a current phase 2 multicenter, double-blind, randomized, placebo-controlled trial of fasudil in ALS patients.\textsuperscript{114} Berberine is a natural isoquinoline alkaloid that acts in part as a Rho kinase inhibitor and affects sigma receptor 1, similar to many antidepressant drugs.\textsuperscript{115} Sulfate salts of berberine readily cross the blood–brain barrier and have been used clinically for their antimicrobial and antifibrotic effects, and promote the integrity of the blood–brain barrier by inhibiting Rho and activating AMPK to promote autophagy.\textsuperscript{116,117} In ALS, berberine has been shown to reverse the processing of insoluble TDP-43 aggregates through the activation of the autophagic degradation pathway by acting as a Rho kinase inhibitor.\textsuperscript{118} Berberine derivatives promote degradation of mutant proteins of ALS in spinal and bulbar muscular atrophy, and has overlapping putative effects on misfolded proteins in amyotrophic lateral sclerosis and frontotemporal-lobar degeneration.\textsuperscript{119}

In regards to lipid-based risk factors in ALS, analyses have demonstrated that higher low-density lipoprotein (LDL) levels are causally associated with an increased risk of developing ALS in both European and East Asian populations.\textsuperscript{120} While there is caution in prescribing statins for patients with myopathies, it is interesting to note that cholestyramine binds bile acids and reduces LDL cholesterol, and is approved by the FDA for the management of dyslipidemias. Interestingly, regarding its potential
use in ALS, cholestyramine binds mold toxins produced by various fungal species.121

**ADDRESSING PHYSICAL TRAUMA**

Biophysical methods, including the use of focused ultrasound (FUS) modalities, have been shown to improve glymphatic dysfunction. For example, it was recently shown that focused ultrasound was able to reduce the amyloid burden and improve cognition in a small cohort of AD patients. Therapeutic FUS has been shown to reduce Aβ plaque in a small group of patients with AD.122 Mechanistically, FUS has been shown to expand the perivascular spaces of the brain to enhance the dispersion of protein aggregates and induce endothelial production of nitric oxide (NO) release to augment microvascular perfusion.123 Potential therapeutics that can improve glymphatic function include assessment and correction of abnormalities through manual therapies, such as atlas orthogonal modalities treatment to relieve chronic cerebrospinal venous insufficiency secondary to mechanical compression of the epidural space.124

**ADDRESSING RESPIRATORY DYSFUNCTION**

Key respiratory muscle dysfunction in ALS involves the diaphragm, accessory muscles of respiration, and bulbar muscles, which can lead to aspiration, diminished airway clearance, ineffective cough, recurrent pulmonary infections, and chronic hypercapnic respiratory failure.125–127 Clenbuterol, a beta-agonist, is currently used in trials for ALS, but common side effects, including tremor and headache and dose-dependent abnormalities of laboratory values including hypokalemia and hypoglycemia, may preclude its long term use.128 Respiratory muscle dysfunction in ALS can be addressed by pharmacological agents such as aminophylline, an FDA-approved drug for the treatment of chronic obstructive pulmonary disease, by acting as an adenosine A2a receptor antagonist.129 Aminophylline induces long-lasting phrenic motor facilitation and may provide an effective therapeutic strategy in the treatment of patients with ventilatory control disorders, such as obstructive sleep apnea, or respiratory insufficiency after a spinal injury or during neurodegenerative diseases.130 In a randomized controlled study in ALS, the administration of aminophylline led to improvements in forced vital capacity, maximal mouth inspiratory and expiratory pressures (\(P_{\text{im}}/P_{\text{em}}\)), and handgrip strength.131

**ADDRESSING PSYCHOLOGICAL FACTORS AND IMPROVING SLEEP**

There are anecdotal reports that lithium is helpful in patients with pseudobulbar palsy, a common co-morbid feature in ALS patients characterized by emotional lability.132 Mechanistically, a further rationale to consider lithium on an empirical basis is its putative effects on RNA metabolism in ALS. Lithium is known to act as an autophagy enhancer by inhibiting GSK-3β and has been shown (in low doses) to reduce Aβ plagues and p-tau levels.133 Lithium also has supportive effects in collapsin response mediator proteins (CRMPs) that act to stabilize microtubules via the enhancement of microtubule-associated protein 1 light chain 3 (LC3) to support autophagy.134–137 Mutations in CRMP interact with RhoA to suppress neurite outgrowth.138 In ALS mouse models, genetic corrections to CRMP2 phosphorylation delayed the progression of motor symptoms in the mutant model.139,140 Lithium regulates the activity of CRMP by inhibiting GSK3β expression, which is upregulated in ALS.141 Lithium administration also has been shown to promote autophagy flux to protect motor neurons in the spinal cord.142,143 Lithium has also been shown to result in a marked increase in LC3-positive vacuoles in mouse models and has shown therapeutic potential in the clinical setting for ALS and its mood disorder-related pathology.144–148

Antidepressants, especially serotonin agonists, may have a protective effect in ALS by acting through sigma 1 receptor (and/or AQP4 receptors) expressed in motor neurons in the brainstem and in spinal motor neurons.149 Activation of sigma 1 receptors is currently being investigated in ALS and Huntington disease, which through the restoration of autophagic flux may be neuroprotective.150,151 Recent studies have demonstrated that defects in SigR1 receptor binding are found in ALS patients.152 Sig-1R agonists have been found to increase Sig-1R expression and translocation to relieve blood–brain barrier dysfunction; meanwhile both Sig-1R knockout and Sig-1R
Antagonists block the neuroprotective functions of the receptor.\textsuperscript{153}

Untreated depression can lead to anorexia and weight loss due to reduced caloric intake, and this reduction in consumption is an indicator of poor prognosis in ALS patients. Motor neurons require vast amounts of energy utilized by the mitochondria and reduced food intake and anorexia may exacerbate muscle catabolism.\textsuperscript{154} As expected, caloric restriction shortened the lifespan of ALS mice models.\textsuperscript{155} Conversely, performed with mutant SOD1\textsuperscript{G93A}, SOD1\textsuperscript{G86R}, and TDP43\textsuperscript{A315T} overexpressing mice, a high-fat diet delayed disease onset and extended survival, and in other studies attenuated motor neuron loss.\textsuperscript{156,157} In a double-blind, placebo-controlled trial, a high-fat hypercaloric diet led to a slower decline in ALSFRS-R scores compared to matched controls.\textsuperscript{158}

Pain is often an under-recognized symptom both in depression and in ALS patients. Palmitoylethanolamide (PEA), an endogenous compound belonging to the family of N-acylethanolamines, has been shown to alleviate pain signals by acting as a cannabinoid CB2 receptor agonist, also demonstrating beneficial effects in a small pilot study for depression.\textsuperscript{159,160} PEA also inhibits the Ca\textsuperscript{2+}-dependent release of glutamate, and as excessive glutamate has been associated with many neurodegenerative diseases, PEA appears to be a potentially strong mechanistically based compound to consider in ALS patients.\textsuperscript{161} In this regard, PEA has been shown to reduce the rate of acetylcholine receptor desensitization at the neuromuscular junction in an average of 70\% in ALS patients.\textsuperscript{162} Based upon these findings, PEA treatment was examined in a small pilot study of 28 ALS patients to receive 50 mg riluzole twice daily plus 600 mg PEA twice daily. No significant reductions in forced vital capacity were detected by pairwise comparisons during the analysis in PEA-treated patients.\textsuperscript{162}

Buspirone is an anxiolytic drug with robust serotonin receptor 1A (Htr1a) agonist activity that acts as a respiratory stimulant through brainstem chemoreflex afferents in central apnea.\textsuperscript{163} The drug has also been shown to reduce hypocapnic central sleep apnea in chronic spinal cord injury patients, in which hypocapnic sleep apnea is a common complication in ALS.\textsuperscript{164} Pharmacologically, buspirone not only acts as a serotonin agonist but also has an affinity for sigma receptor 1 (SigR1), which plays an important role in regulating motor neuron function and autophagy – processes that are critical in ALS.\textsuperscript{165} In randomized double-blind, placebo-controlled multicenter studies (phase III) with xaliproden – a 5-HT\textsubscript{1A} receptor agonist – lead to improvements in vital capacity that are commonly reduced during sleep in ALS patients.\textsuperscript{166}

Ashwagandha extract has been shown to result in significant improvement in overall sleep compared to placebo in the dosage of ≥600 mg/day.\textsuperscript{167} Regarding its potential putative effects in neurological and inflammatory disorders, withaferin A – a bioactive constituent from \textit{Withania somnifera} – has been shown to mitigate inflammatory cytokines, including nuclear factor kappa-B (NF-\kappa B) and tumor necrosis factor-alpha (TNF-\alpha), as well as diminish the expression of pro-fibrotic proteins.\textsuperscript{168} Withaferin A also induces heat shock proteins that are instrumental in promoting protein folding.\textsuperscript{169} Oral analogs of withaferin-A act as an antagonist of NF-\kappa B and have been shown to reduce TDP-43 proteinopathy in the brain and spinal cord of transgenic mice expressing human TDP-43 mutations and restored the synthesis of neurofilament proteins.\textsuperscript{170}

Activation of melatonin MT\textsubscript{1} and MT\textsubscript{2} receptors exerts neuroprotective effects by inhibiting NF-\kappa B activation, protecting neurons from lipopolysaccharide toxicity.\textsuperscript{171} In animal models of sepsis, melatonin administration inhibited the disruption of BBB permeability by attenuating microglial toll-like receptor 4/NF-\kappa B signaling.\textsuperscript{172} Melatonin also plays a key role in autophagy by the regulation of autophagy mediated by the phosphorylation of AMPK.\textsuperscript{173} Retrospective analysis regarding the impact of melatonin on progression and overall survival of ALS revealed that melatonin was shown to have a significantly decreased annualized hazard death rate, slowed the rate of functional decline, and change in predicted forced vital capacity score compared with the non-melatonin users.\textsuperscript{174}

\textbf{CONCLUSION}

In summary, ALS has recently been recognized as a systems biology disorder, given that neurodegenerative changes occur not only in motor neurons but also in non-neurological tissue as well, including the
vascular system. This may account for significant co-morbidities related to sleep disorders, immune senescence, MRI changes in both white and gray matter, and pulmonary dysfunction. Both genetic and non-genetic factors contribute to the risk of developing ALS. Sporadic risk factors based upon epidemiological and anecdotal case studies point to a predilection for recurrent infections and head/neck trauma as possible contributing factors. These environmental insults converge on autophagy, an evolutionarily conserved process required to remove the pathological aggregates associated with ALS. Addressing ALS from a systems biology approach – including addressing sleep disorders, pain, musculoskeletal dysfunction, and breathing capacity – may yield unexpected synergistic effects in treatment beyond a single monotherapeutic agent.

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COMPETING INTERESTS

The authors declare they have no competing interests.

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AUTHORS’ CONTRIBUTIONS

J.L. collated all of the content in the manuscript. M.H. contributed to the preparation and revision of the manuscript. J.L. and M.H. contributed to the conception and interpretation of content, and are in agreement with all aspects of the work. All authors read and approved the final manuscript.
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