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The Role of Astrocytes in the Pathogenesis of Amyotrophic Lateral Sclerosis

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Abstract

Astrocytes are glial cells that play a crucial role in providing a supportive environment for neurons, mainly by cell-cell interactions mediating numerous physiological and biochemical functions in the central nervous system (CNS). Astrocytes are also critical for intercellular signaling in the neurovascular unit. Although numerous reports have detailed the complex effects of astrocytes upon neurons in neurodegenerative disease, whether or not astrocytes act as initiators or contributors in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) remains unclear. A broad overview of astrocytes' link to motor neuron degeneration in ALS, as well as specific functions of astrocytes within the neurovascular unit is presented. This review summarizes current knowledge of the astrocyte's role in disease pathogenesis and discusses the potential of the astrocyte as a target for future ALS therapies.

Keywords: Amyotrophic Lateral Sclerosis; Astrocytes; Motor Neurons; Neurovascular Unit; Therapies

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease involving both upper (motor cortex and brainstem) and lower level (spinal cord) motor neuron death [1]. ALS is predominantly, 90-95%, sporadic with the remaining 5-10% of cases considered genetically linked (familial) [1]. Although the specific mechanisms responsible for sporadic ALS remain a mystery, within familial ALS cases, approximately 20% result from missense mutations in the genes coding for Cu/Zn Superoxide Dismutase (SOD1) [1, 2]. The clinical presentation and underlying pathology of sporadic and familial ALS are similar, and current treatment options for ALS patients are limited and mainly supportive. The only FDA approved drug to treat ALS is riluzole.

ALS pathogenesis is complicated by the diffuse nature of motor neuron degeneration and by the complexity of associated intrinsic and extrinsic factors. Evidence from various investigative studies established that ALS related factors include: glutamate excitoxicity, mitochondrial dysfunction, oxidative stress, glial cell pathology, impaired axonal transport, protein aggregations, immune response, neurotrophic factor deficits, and neuroinflammation [3-11].

Relatively recent, compelling evidence of structural and functional alterations in the Blood-Brain Barrier (BBB) and Blood-Spinal Cord Barrier (BSCB) has been demonstrated in both patients and animal models of ALS [12-19]. This vascular pathology, including impairment of all neurovascular unit components in the brain and spinal cord, has been recognized as a key factor for identifying ALS as a neurovascular disease [19, 20]. The astrocyte, part of the neurovascular unit and closely associated with motor neuron degeneration, seems likely to play a significant role in ALS-related barrier pathology.

The interaction between neurons and astrocytes is largely dependent on the CNS microenvironment. In healthy tissue, the primary astrocyte functions are neuroprotection and regulation of homeostasis. However, if the microenvironment is pro-inflammatory, astrocytes can promote degeneration of neurons. Astrocytes have an established role in the regulation of neurometabolic function, including the uptake and release of various neurotransmitters, specifically within the synaptic cleft [21]. Glutamate excitotoxicity has been reported in ALS as well as other neurodegenerative diseases, however causative mechanisms are yet unknown [6]. Astrocyte expression of glutamate transporters illustrates the close relationship between astrocytes and glutamate excitotoxicity. Interactions that lead to the inactivation of Excitatory Amino Acid Transporter 2 (EAAT2) within astrocytes also contribute to the degeneration of motor neurons [5]. Reactive astrocytes can obstruct the spread of hazardous molecular debris and/or generate a pro-inflammatory microenvironment. Unfortunately, excessive astrogliosis can further promote neurodegeneration [11]. Expression of various chemokines by astrocytes may propagate neurodegeneration through activated microglia leading to neuroinflammation [22].

With regards to the neurovascular unit, astrocyte end-feet interact with the capillary lumen and regulate BBB/BSCB integrity. Astrocytic intracellular calcium concentration might regulate neuronal activity leading to vasomotion (oscillation) of cerebral arterioles [23]. Release of specific factors, such as Vascular Endothelial Growth Factor A (VEGF-A), also facilitates the interaction between astrocytes and the BBB/BSCB by increasing microvascular permeability [24]. This implies that astrocytes may have a substantial role in the regulation of vascular permeability and oscillation. Aquaporin4 (AQP4) expression within astrocytes can lead to perivascular edema as well as to neuroinflammation, leading to neurodegeneration [25]. Astrocytes from $SOD1^{G93A}$ transgenic mouse and rat models of ALS showed overexpression of AQP4 and downregulation of potassium channels, which affect motor neuron function [26]. In light of multiple astrocyte functions, a review of such mechanisms with regards to ALS pathology may promote a better understanding of disease-related neurodegeneration and may facilitate the identification of potential therapeutic options for this disease.

Astrocytes and Motor Neuron Degeneration

The specific interactions between astrocytes and motor neurons that might contribute to the motor neuron degeneration in ALS have been reported in numerous studies. Although multiple factors relate to ALS pathogenesis, glutamate excitotoxicity has been mainly linked to disease progression [6, 27]. Astrocytes surrounding the synaptic clefts of neurons remove excess glutamate, providing an important neuroprotective function [28]. Astrocytes are also involved with synapse remodeling during CNS development and after trauma [28]. Glutamate transporters within the brain that are specific to astrocytes include EAAT1 and EAAT2; the latter has been shown to be of crucial importance by multiple studies [5, 29, 30]. Studies of knockout mouse models of EAAT2 revealed that the transporter promotes epilepsy and cell death [31]. A cross between an ALS mouse model and mice overexpressing EAAT2 led to postponement of disease onset, suggesting that loss of EAAT2 aggravates motor neuron degeneration within ALS [29]. Astrocytes can directly impact glutamate excitotoxicity and interact with pathways linked to motor neuron degeneration by regulation of EAAT1 and EAAT2. Screening of sporadic ALS patients found abnormal EAAT2 mRNA, suggesting that down regulation of this transporter was related to defective mRNA [30]. The EAAT1 and EAAT2 abnormalities seen in ALS pathology would inhibit glutamate clearance by astrocytes, promoting excite toxicity and neurodegeneration [5, 30]. Astrocytes thus play a pivotal role in glutamate regulation and have a role in the degeneration of motor neurons through glutamate excitotoxicity.

Motor neuron degeneration within ALS is not the only consequence of glutamate excitotoxicity. Chronic glutamate excitotoxicity can lead to astrogliosis and impairment of mitochondrial energy metabolism [32]. Also, elevated concentrations of Tumor Necrosis Factor alpha (TNF-α) have been found in the plasma of sporadic ALS patients, suggesting involvement of the TNF pathway in ALS [33]. Astrocytes have the unique ability to respond to inflammatory stress through their resistance to apoptosis triggered by the FAS ligand and the TNF Related Apoptosis Inducing Ligand (TRAIL) [34]. Calcium/calmodulin-dependent protein kinase II (CaMKII) as well as its interactive pathway allow the astrocyte to resist such apoptosis, but lead to the formation of reactive astrocytes [34]. Reactive astrocytes lead to astrogliosis, which is mediated by TNF- α [35]. Astrogliosis has been noted in the grey and white matter of the brain in both familial and sporadic ALS [36, 37]. Astrogliosis is another important mechanism by which astrocytes interact with motor degeneration, as reactive astrocytes primarily surround neurons undergoing degeneration [11]. The presence of reactive astrocytes correlates with increases in microglial infiltration and production of nitric oxide synthase, both known promoters of neuroinflammation and neuronal degeneration. However, increased proliferation of astrocytes furthers negative consequences; most notably increased pro-inflammatory cytokines and nitric oxide concentrations, and indirectly promotes the degeneration of neurons [11, 22].

Analysis of astrocytes cultured from mice with the $SOD1^{G93A}$ mutation reveals the close relationship between neuroinflammation and motor neuron degeneration. Pro-inflammatory cytokine upregulation, increased oxidative stress and astroglial morphological changes are seen in mice with mutant SOD1 [38]. Astrocytes regulate the microenvironment by activating surrounding microglia, thus having an indirect degenerative impact on motor neurons. This inflammatory microenvironment and the subsequent astroglial morphological changes can lead to the infiltration of T-cells into the spinal cord of ALS patients and induce motor neuron damage [39]. Furthermore, *in vitro* studies have shown that extracellular mutant SOD1 induced microglial-mediated motor neuron damage [40]. The positive correlation between microglia activation and neurotoxicity suggests that microglia activation is needed to intensify the toxicity of mutant SOD1 to motor neurons. However, investigations have shown that removal of proliferating microglia does not impact motor neuron death [41]. This suggests that a cell, other than the microglia, is responsible for the motor neuron death, making astrocytes a likely culprit. As reactive astrocytes have been shown to induce a microenvironment leading to microglia activation and neuroinflammation, this reveals another mechanism in which astrocytes likely lead to ALS motor neuron degeneration. In light of such knowledge, treatment options that look to inhibit reactive astrocytes or alter astrocytic impact on the microenvironment may prove beneficial in the treatment of ALS.

A comprehensive study showed that removal of mutant SOD1 genes exclusively from astrocytes and/or microglia leads to slower disease progression and a doubling of the lifespan of G93A SOD1 mice [42]. Interestingly, removal of mutant type SOD1 genes from 30-50% of motor neurons delayed disease onset, but lifespan was not increased in these ALS mice. The authors suggested that the SOD1 mutation in motor neurons has the main role in the onset of ALS, whereas astrocytes and microglia are leading players in promoting the disease progression [42]. Astrocytes are also known to regulate the immunity within the nervous system, although they do not share the same lineage as other immune cells, like microglia. Astrocytes with mutant SOD1 release factors that promote toxic effects on motor neurons by the B-Cell CLL/Lymphoma 2-associated X protein (Bax)-dependent cell death pathway; however, it should be noted that this pathway was found to only affect spinal motor neurons [43]. Misfolded aggregates of the mutant SOD1 were found within astrocytes and other glial cells throughout the spinal cord, brainstem, and motor cortex of ALS patients [10]. Thus, mutant-SOD1-related toxic effects can engender an inflammatory microenvironment promoting motor neuron degeneration in ALS. Additionally, this toxic environment also disrupts the astrocyte and vascular interactions in the neurovascular unit.

Astrocytes and the Neurovascular Unit

Recent studies have highlighted the fact that BSCB/BBB is altered in ALS and that repair of this barrier should be a focus of future treatment options [12-19]. Physiologically speaking, the BBB/BSCB plays an essential role in regulating CNS homeostasis by controlling the entry and exit of various substances through the barrier system. Astrocytic end-feet surround pericytes and endothelial cells that are tightly bound around the blood vessel by

tight junction proteins, while the other astrocyte processes interact at various locations on the neuron. The intermediate location of astrocytes allows for bidirectional communication between the neuron and the capillary, and highlights the importance of astrocytes as a key component of the neurovascular unit.

Astrocytic communication within the neurovascular unit is vitally dependent on cellular concentrations of calcium. Neuronal activity induces calcium changes in astrocytes, which mediate vasomotion within the arterioles [23, 44]. Increased TNF-α in transgenic mice showed a resulting disruption of the BBB either by astrocyte dysregulation or by an inflammatory mechanism triggered by the astrocyte [45]. Therefore, there is a strong link between inflammation, dysregulation of astrocytes and disruption of the BBB. This specific link is supported by a report showing that reactive astrocytes can lead to the breakdown of the BBB through the expression of VEGF-A, and demonstrating the impact of VEGF-A on tight junction proteins Claudin 5 (Cldn5) and Occludin (Ocln) [24]. Furthermore, AQP4 is overexpressed in ALS animal models and can lead to further impairments within the neurovascular unit [15]. Analysis of the ultrastructure of the brainstem and spinal cord segments of an ALS mouse model showed disconnection of astrocyte end-feet from capillaries, hypothesized to be caused by astrocyte damage, which led to AQP4 dysfunction and perivascular edema formation [12]. Astrocytic damage and dysfunction can lead to an inability to maintain potassium homeostasis as well as other ionic conditions resulting in BBB/BSCB damage [15, 26]. Importantly, downregulation of potassium channel subunit Kir4.1, which is seen in ALS animal models, was shown to inhibit glutamate uptake by Kir4.1 RNA interference (RNAi) [46]. Damage of BBB/BSCB, alteration of astrocyte activities and neurodegeneration – through the restriction of glutamate uptake/clearance – have been shown to occur due to AQ4 overexpression and down regulation of Kir4.1 [26, 46].

Also, elevated IgG and albumin levels in the CSF of ALS patients may stem from an altered blood-CSF barrier and lead to motor neuron degeneration [47, 48]. A decrease in tight junction proteins within endothelial cells has been reported in mutant SOD1 mouse models prior to motor neuron degeneration [16]. Sporadic and familial ALS patients also show a decrease in mRNA expression of tight junction proteins such as zona occludens 1 (ZO-1), Ocln and Cldn5, additional evidence that the neurovascular unit should be a focus of future ALS studies [49]. Albumin activates production of Matrix Metalloproteinase 9 (MMP9) within astrocytes and consequentially increases astrocyte production of reactive oxidative species; furthermore, MMP9 was shown to disrupt the neurovascular unit [50, 51]. Moreover, the severity of ALS inversely correlates with gray matter perfusion, again suggesting that the pathology of ALS is closely associated with dissociation of neurovascular components [52]. Thus, impaired BBB/BSCB, evidenced by decreases in tight junction proteins [24,49], increases in abnormal infiltrates in the CSF [47,48], and increases in oxidative species [50,51] can impact and propagate motor neuron degeneration in ALS [11,17,18,22,39,45]. In light of such evidence, it is important to further explore the specific role of astrocytes within the neurovascular unit in development of beneficial treatments for ALS.

Potential Therapeutic Strategies

As ALS is a multifactorial disease, tailored therapeutic strategies should also be multifaceted. ALS, like many neurodegenerative diseases, has a pathology that has been extensively studied but the mechanistic underpinnings are still poorly understood. Unfortunately, the one available treatment for patients with ALS is the therapeutic drug Riluzole, which provides only minimal benefit. Having reviewed the specific effects of astrocytes in regards to motor neuron degeneration, it seems that astrocytes could be key to developing a suitable treatment option for ALS.

Glutamate excitotoxicity, as a result of decreased glutamate transporter expression, was observed in the astrocytes of mutant-SOD1 mouse models, thus a valuable therapeutic option for ALS might be targeting glutamate transporter systems within astrocytes. Recent findings have shown that antibiotics of the β-Lactam family increase the expression of EAAT2 and lead to delayed disease progression and increased survival in mouse models [53]. Unfortunately, the clinical trial of these antibiotics in ALS patients was stopped at Phase III due to inability to reach the specified efficacy criteria [54]. Although a disappointing result, hope remains that increasing glutamate transporter expression within astrocytes may be a potential treatment option. Electroacupuncture was also shown to upregulate EAAT1 and EAAT2, a mechanism which potentially explains its analgesic effect [55]. Perhaps electroacupuncture could be used as a

rehabilitative treatment option for ALS patients to delay disease progression. The transmembrane protein B-Klotho has also been shown to upregulate EAAT1 and EAAT2 within oocytes and could be another treatment option to rectify the glutamate excitotoxicity seen in many neurodegenerative diseases [56]. Up-regulating decreased astrocyte glutamate transporter expression seems a promising area for ALS research, considering the key role of astrocytes in motor neuron degeneration. In addition, widespread astrogliosis, prolonged inflammation, and the damaged BBB/BSCB also need targeting in any successful ALS therapy.

Astrogliosis is widespread in CNS tissues in ALS. Although reactive astrocytes might have neuroprotective functions, they also have neurotoxic effects [36]. The interactions of reactive astrocytes with glutamate uptake, reactive oxygen species and production of pro-inflammatory cytokines make astrocytes a likely therapeutic target. Indeed, delayed manipulation of the sphingosine-1-phosphate signaling pathway following induced stroke within mouse models showed a reduction in reactive astrogliosis and improved neurological outcome [57], demonstrating potential therapeutic benefits which might be applied in an ALS therapeutic strategy. Astrogliosis occurs in locations proximal to motor neuron degeneration, making astrocytes a prime therapeutic target. Specifically, reactive astrogliosis has been shown to concentrate in close proximity to corticospinal tracts (lateral funiculus) and laminae V to VII in ALS patients [58]. Astrocytes from ALS patients, both sporadic and familial, co-cultured with motor neurons lead to motor neuron degeneration; however, lentiviral knockdown of SOD1 from the astrocytes alleviated this neuron degeneration [59]. Transplantation of glial restricted precursors not only slowed disease progression but also demonstrated a neuroprotective effect in decreasing the overall loss of EAAT2; again, the value of astrocyte-targeted treatment options is highlighted [60]. Anti-TNF treatment reduced astrogliosis and decreased BBB permeability in irradiated mice [61]. This finding positively correlates with study results showing that TNF-α negatively impacts BBB status and increases astrogliosis [45], suggesting that anti-TNF treatment might show benefit in ALS. Low doses of 17 β-estradiol in treatment of mice modeling spinal cord injury were also found to reduce astrogliosis and increase angiogenesis. Combined with the increased expression of neuroprotective factors, this approach led to improved limb function [62]. Treatment options such as those previously described, that reduce astrogliosis while increasing angiogenesis, seem ideal candidates for ALS. Reduced astrogliosis and increased angiogenesis could be accomplished via stem cell administration, and also by providing necessary neuroprotective growth factors that need to cross the barrier to the CNS [63]. However, several ALS clinical trials of various growth factors to combat neurodegeneration were halted due to the inability or restricted ability of these factors to cross the BBB [64]. Rather than crossing the dysfunctional BBB/BSCB and altering the microenvironment within the CNS parenchyma, ALS therapy treatments could focus on repairing the dysfunctional BBB through astrocytes.

Astrocytic end-feet surrounding capillaries and pericytes, endothelial cells and their tight junctions, comprise the blood-CNS barrier, which allows glucose efflux through specific transport mechanisms and regulates certain metabolic interactions that provide neurons with vital substrates [65]. Astrocytes, which lack connections to capillaries through their end-feet, as seen within disrupted neurovascular units of ALS patients and animal models of disease, could thus exacerbate neurodegeneration by not providing motor neurons sufficient energy, leading to neuronal death. Such disruption might also have a cascading effect leading to astrocyte degeneration and consequent increases in glutamate excitoxicity. Targeting the disrupted attachment of astrocyte end-feet, as well as loss of astrocyte glutamate uptake, could be a therapeutic option for ALS. Hypertonic saline is currently used as a treatment of cerebral edema since it inhibits AQP4 in astrocytes and could thus be used as a possible treatment option at early stages of ALS in the hope of preventing any detrimental cascade of events. The neuroprotective molecule, guanosine, has been shown to specifically alleviate deficiencies in potassium conductance by promoting the up-regulation of Kir4.1 channels [66]. Guanosine can inhibit excitotoxicity by using the phosphoinositide-3-kinase (PI3K)/AKT pathway to prevent glutamate release, further suggesting it as a possible treatment option for ALS [67, 68].

Another potential therapeutic strategy for ALS would be restoration of the microenvironment, which is altered at early stages of the disease by astrogliosis and BBB/BSCB damage, and at late stages by motor neuron degeneration and inflammation.

These adverse effects destabilize the CNS ionic homeostasis and lead to a cascade of events that severely impacts the resident cells of the CNS. Stem cell therapy could prove a viable treatment option by restoring the necessary levels of growth factors and signals in order to delay disease progression. It has been shown that administration of stem cells such as glial restricted precursors decreased motor neuron loss in a mouse model of ALS [60].

Conclusion

There is strong evidence suggesting that astrocytes play an important role in ALS pathogenesis by inducing motor neuron degeneration and neurovascular unit damage. In this review, astrocytes are highlighted as potential cellular targets for the development of therapeutic strategies for ALS. Neurovascular damage seems to be an initial pathological event and points to the fact that ALS may be considered as a neurovascular disease [19, 20]. This neurovascular dysfunction is hypothesized to be a significant influence on motor neuron degeneration [17-19]. Also, inhibited glutamate clearance [5,30], the overexpression of AQP4, downregulation of Kir4.1, and the increase in unwanted infiltrates across the BBB/BSCB, all point to astrocytes being key cells in the disease progression and in terms of neurovascular dysfunction [15,26,46-48]. Stem cell therapies to replace damaged astrocytes or drug treatments to stabilize the favorable microenvironment for motor neurons are promising therapies and likely candidates for future ALS clinical trials. Studying astrocyte links with glutamate excitotoxicity and inflammatory processes illuminates the close relationship between astrocytes and neurodegeneration. Multiple studies suggest a vital and central role for astrocytes in ALS progression by impacting the neurovascular unit and promoting neurodegeneration [14, 16-20, 26, 46]. Future ALS research should investigate specific treatment options to alleviate astrocyte-related damage.

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