Mechanisms of glutamate toxicity in multiple sclerosis: biomarker and therapeutic opportunities

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Research advances support the idea that excessive activation of the glutamatergic pathway plays an important part in the pathophysiology of multiple sclerosis. Beyond the well established direct toxic effects on neurons, additional sites of glutamate-induced cell damage have been described, including effects in oligodendrocytes, astrocytes, endothelial cells, and immune cells. Such toxic effects could provide a link between various pathological aspects of multiple sclerosis, such as axonal damage, oligodendrocyte cell death, demyelination, autoimmunity, and blood–brain barrier dysfunction. Understanding of the mechanisms underlying glutamate toxicity in multiple sclerosis could help in the development of new approaches for diagnosis, treatment, and follow-up in patients with this debilitating disease. While several clinical trials of glutamatergic modulators have had disappointing results, our growing understanding suggests that there is reason to remain optimistic about the therapeutic potential of these drugs.

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Introduction

Multiple sclerosis is a chronic disease of the CNS that usually first manifests between ages 20 and 40 years, and is estimated to affect about 2·5 million people worldwide. The disease causes substantial disability that is associated with considerable economic burden.[1] The clinical pattern of multiple sclerosis ranges from inflammatory relapsing-remitting disease (the most frequent initial presentation) to chronic progressive disease without flairs and remissions.[2] Mechanisms implicated in the pathogenesis of multiple sclerosis include autoimmunity, inflammation, demyelination, and neurodegeneration.[3] Improved understanding of these mechanisms will allow the development of new therapeutic strategies to slow or stop progression of relapsing-remitting forms of multiple sclerosis and to develop effective disease-modifying treatments for the progressive forms of the disease. Additionally, patients and clinicians would gain from innovative diagnostic tools that could predict the different phases of multiple sclerosis to enable early intervention, and improved follow-up and clinical trials.

Histopathological studies have implicated dysregulation of the glutamatergic system in the pathogenesis of multiple sclerosis, and animal studies have helped to decipher the mechanisms by which excessive glutamate might contribute to the disease process. Several clinical trials have been done to assess the efficacy of the modulators of the glutamatergic system in multiple sclerosis, but none has so far provided evidence of substantial clinical benefits. However, on the basis of results of these trials and improved understanding of the therapeutic promise of glutamatergic modulators in multiple sclerosis, new clinical trials are underway.

Here we discuss the role of glutamate toxicity in multiple sclerosis and how glutamatergic pathways might be targeted to improve diagnosis and treatment. We consider the lessons that could be learned from clinical trials of glutamatergic modulators in multiple sclerosis.

Sources of excess extracellular glutamate

Several mechanisms might contribute to extracellular accumulation of glutamate in white matter and grey matter.

Release by inflammatory cells

Inflammatory cells are a source of extracellular glutamate in the context of neuroinflammation. A substantial source of glutamate from inflammatory foci was initially suggested to be neutrophils,[4] but monocytes and macrophages,[5] microglia,[6] and dendritic cells[7] are now viewed as being the most important sources in multiple sclerosis. These cells release glutamate through the cysteine/glutamate antiporter Xc^- (figure 1),[5–9] the expression of which increases following cell activation.[5,7] Xc^- is upregulated in peripheral blood leucocytes, optic nerve, and spinal cord samples from human beings with multiple sclerosis, and in spinal cord samples from animals with experimental autoimmune encephalomyelitis, a model of multiple sclerosis.[5] Pharmacological inhibition or genetic ablation of this antiporter reduces disease severity in animal models;[6] these findings and data from in-vitro studies[6] suggest that glutamate released from activated microglia is sufficient to cause substantial damage to myelin.

Glutamate is released when dendritic cells contact T cells,[7] which suggests that glutamate produced by dendritic cells has a role in T-cell activation induced by antigen presentation. Macrophages, dendritic cells, and microglia are all thought to function as specialised antigen-presenting cells in multiple sclerosis,[10] and the effects of glutamate during antigen presentation might involve macrophages and microglia as well as dendritic cells. Finally, T cells can, in turn, induce the release of glutamate from antigen presenting cells,[6] which is speculated to result in a positive feedback loop (figure 1).

In addition to Xc^--mediated glutamate release, dendritic cells store glutamate in specific vesicles for fast Ca^{2+}-dependent release.[11] Vesicular release of glutamate has been shown in the context of thymic selection;[12] however, its relevance to neuroinflammation needs further exploration.

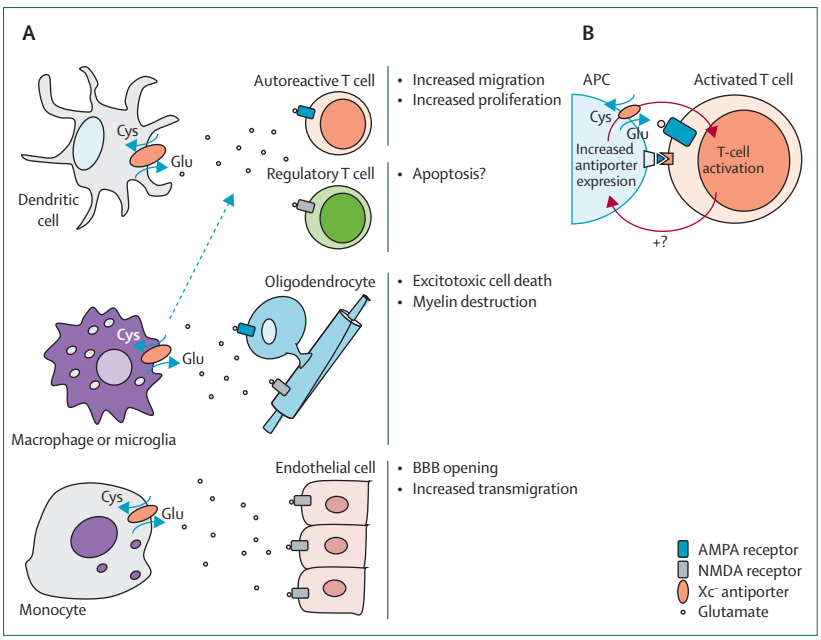

***Figure 1*: Dysfunction of the glutamatergic system in inflammatory and immune cells**
(A) In multiple sclerosis, dendritic cells, macrophages, and monocytes release glutamate via the Xc^-cysteine/glutamate antiporter , which is upregulated in activated cells under inflammatory conditions. At the neurovascular unit, neurons, dendritic cells, astrocytes (and possibly macrophages and microglia) release glutamate, leading to increased migration and proliferation of autoreactive T cells via stimulation of AMPA receptors. Increased glutamate concentrations could induce apoptosis of regulatory T cells via activation of NMDA receptors. Glutamate released by macrophages or microglia might induce excitotoxic cell death in oligodendrocytes via AMPA activation[8] and myelin damage via NMDA receptors.[9] Glutamate release by circulating cells, such as monocytes, might also contribute to BBB dysfunction and increased leucocyte transmigration by activation of NMDA receptors on endothelial cells. (B) During antigen presentation, glutamate is released by APCs via the Xc^- antiporter. The activated T cells in turn induce the release of glutamate by APCs, which is speculated to lead to a positive-feedback loop. APC=antigen-presenting cell. BBB=blood–brain barrier.

The production of glutamate by inflammatory cells, especially monocytes, is important for their infiltration into the CNS. Activated monocytes release glutamate via upregulation of Xc^-,[5] the inhibition of which is accompanied by a reduced infiltration of peripheral immune cells into the CNS.[6] Glutamate released from monocytes might also activate glutamate receptors expressed on endothelial cells to facilitate their infiltration. Glutamate produced by inflammatory cells, in particular microglia that have been activated by inflammatory mediators released by T cells, also participates in the demyelination process, possibly by inducing excitotoxic death in myelinating oligodendrocytes.[6]

Increased glutamatergic transmission

Excessive presynaptic release in grey matter is one explanation for increased synaptic concentrations of glutamate. A parallel series of events might occur in white matter. Increased expression of voltage-gated sodium channels (VGSCs) in demyelinated axons, as an attempt to restore conduction,[10,13,14] might contribute to axonal depolarisation and high intra-axonal Na^+ concentrations. Overexpression of sodium-channel subunits $Na_v1.2$ (also known as SCN2A) and especially $Na_v1.6$ (SCN8A), which leads to persistent non-inactivating conductance,[13,15] would result in excessive Na^+ accumulation in axons. This effect, coupled with compromised mitochondrial function[14] and reduced Na^+/K^+/ATPase activity,[15] would further exacerbate axonal depolarisation and Na^+ loading, and would promote glutamate and glycine release via reverse operation of Na^+-dependent transporters. Vesicular release of glutamate at the axomyelinic synapse is also possible.[10] These changes might lead to excessive concentrations of glutamate in white matter.

Inflammatory cytokines might be responsible for the quantitative changes in the expression of VGSC. The proinflammatory cytokine TNFα increases VGSC expression and the associated Na^+ current in cultured neurons.[16] Conversely, the anti-inflammatory cytokine interleukin 10 reverses the upregulation by TNFα.[17] The inflammatory environment of the CNS typical of multiple sclerosis might, therefore, affect the concentration of intra-axonal Na^+ and the subsequent release of synaptic and axonal glutamate. In tissues from patients with multiple sclerosis, expression of $Na_v1.6$ is increased on damaged axons[18] located within plaques with T-cell infiltrates and activated microglia.[19] These data support the importance of inflammatory processes in the regulation of VGSC expression. Finally, inflammatory cytokines might also affect VGSC expression at axon terminals, and thereby directly modulate presynaptic release of glutamate.

Na^+ content is increased in the brains of patients with relapsing-remitting multiple sclerosis, as demonstrated by sodium magnetic resonance spectroscopy (MRS).[20] Na^+ concentrations are higher in acute and chronic lesions than in normal-appearing white matter of patients, which in turn has higher concentrations than the white matter of healthy controls.[20] Thus, the dysregulation of Na^+ homoeostasis seen in animal studies could be relevant to human pathology. The increased concentration of Na^+ could depolarise axon terminals, leading to presynaptic (grey matter) or axomyelinic (white matter) release of glutamate and subsequent toxic effects. However, whether sodium-channel blockers are efficacious in the treatment of multiple sclerosis is debated.[21,22]

Altered glutamate uptake and release by glial cells

Altered glial glutamate transport contributes to increased extracellular concentrations of the neurotransmitter in the brains of people with multiple sclerosis. The glial glutamate transporters EAAT1 and EAAT2 (also known an SLC1A3 or GLAST and SLC1A2 or GLT-1, respectively) are expressed and functional in astrocytes, microglia, and oligodendrocytes.[8] Expression is decreased in oligodendrocytes around active lesions in white matter of patients with primary or secondary progressive multiple sclerosis.[23,24] In animal studies, downregulation of EAAT1 and EAAT2 during experimental autoimmune encephalomyelitis[25–28] is associated with altered glutamate uptake, predominantly in astrocytes. However,