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REVIEW ARTICLE

Taming Glutamate Excitotoxicity: Strategic Pathway Modulation for Neuroprotection

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Abstract Much work has been carried out in recent years showing that elevated glutamate levels in the extracellular environment of the central nervous system play a pivotal role in neurodegeneration in acute CNS injuries. With the elucidation of the mechanism governing glutamate excitotoxicity, researchers are devising therapeutic strategies to target different parts of the pathway which begins with glutamate accumulation and ultimately results in neuronal cell death. In this article, we review some of the major classes of agents that are currently being investigated and highlight some of the key studies for each. Glutamate scavenging is a relatively new approach that directly decreases glutamate levels in the brain, thus preventing excitotoxicity. Nitric oxide inhibitors and free radical scavengers are more well-studied strategies that continue to yield promising results.

Key Points

Glutamate excitotoxicity plays a central role in cell death during acute neuronal injury.

Glutamate scavenging and reduction is a novel approach to attenuating glutamate excitotoxicity.

Nitric oxide inhibitors and free radical scavengers have shown promising results in animal models but results are mixed in human trials.

1 Introduction

Glutamate is an abundant amino acid established as the most essential neuromodulator of the nervous system [1]. It is the brain's most prominent excitatory neurotransmitter and plays a key role in memory formation and learning. Over the years, studies have demonstrated its central involvement in mechanisms of neuronal death in different brain insults such as traumatic brain injury (TBI), hemorrhage, and ischemia [2–4]. Glutamate excitotoxicity occurs when the neurotransmitter's homeostatic balance is disrupted and levels become elevated in the extracellular fluid [5, 6]. Because of its connection in numerous types of brain injuries, many experiments have assessed how modulation of glutamate and its receptor pathways can play a role in formulating new neuroprotective agents [7]. The ultimate hope is to find a way to slow down, prevent, or stop cell death and thereby preserve neuronal function in disease processes.

Two main events have demonstrated a significant role in neuronal injury: the overstimulation of glutamate receptors

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in the brain and free radical injury. Glutamate receptors are classified as either ionotropic or metabotropic. Ionotropic receptors directly activate ion channel when glutamate binds and are subclassified into NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) or kainate receptors [6]. Metabotropic receptors act more slowly through the activation of G-proteins and a subsequent multitude of pathways. Glutamate excitotoxicity has been shown to cause massive Ca^{2+} influx in the neuronal cells [8, 9], which in turn activates processes (proteases, lipase, nitric oxide synthase [NOS]) that result in irreversible cellular damage [10]. In acute brain processes such as stroke, bioenergetics failure leads to the dysfunction of the Na^{+}/K^{+} ATPase and an eventual disruption of glutamate transport [11]. Additionally, programmed cellular death resulting from irreversible hypoxic injury leads to the release of intracellular glutamate contents.

Via these mechanisms, studies have targeted glutamate excitotoxicity in search of a solution for acute brain insults. Over the last decade, glutamate receptor modulators have emerged as potential therapeutic modalities. Although some receptor modulators showed promise in animal studies, they have thus far failed to demonstrate efficacy in human trials [7, 12]. This has led to a growing interest in blood glutamate scavengers along with other agents that target sites downstream of the receptor in the hope of developing efficacious drug modalities. This review of the literature will explore different therapeutic strategies and their potential as viable options in the treatment of glutamate-induced excitotoxicity.

2 Mechanism of Glutamate Excitotoxicity

Neuronal cell death in the CNS may be classified broadly as physiologic and excitotoxic [13]. Physiologic cell death incorporates apoptotic pathways involving cysteine proteases known as caspases, activator proteins such as Apaf-1, and mitochondrial-associated proteins such as the Bcl-2, and plays a major role in CNS development as well as disease states [14]. On the other hand, excitotoxic cell death, the main focus of this review, is primarily a pathologic process and involves glutamate and its receptors. The mechanisms of glutamate excitotoxicity have been well-studied in both animal models and humans. Although the precise genes and proteins involved are still being elucidated, we have achieved some general understanding of the major pathways that glutamate contributes to neuronal damage [2, 15]. The disruption in glutamate homeostasis is implicated in both acute CNS injuries such as stroke or trauma, as well as in chronic neurodegenerative disorders, including Alzheimer's disease (AD), multiple sclerosis, amyotrophic lateral sclerosis and Parkinson's disease [16–19]. In the following, we discuss mechanisms and therapies of excitotoxicity related predominantly to acute CNS injuries.

Whereas extracellular glutamate levels are increased during neuronal insult, in normal physiological conditions, the high concentrations of neurotransmitter in the brain are stored intracellularly. The concentration of extracellular glutamate is tightly regulated to maintain physiological concentrations through sodium-dependent transporters [5]. Reuptake of glutamate from synaptic junctions after neuron excitation normally involves transporters on nerve terminals and astrocytes, which binds and sequesters the neurotransmitter for processing and recycling [20]. When extracellular concentrations become elevated, sodium-dependent active transport occurs on the antiluminal surface of brain capillary endothelial cells to transfer glutamate from the extracellular fluid [15]. Glutamate accumulates in the endothelial cells to a concentration that exceeds plasma levels, during which it is moved via facilitated diffusion through the luminal side into the blood stream. In this way, the endothelial regulation of CNS glutamate concentration can occur despite unfavorable concentration gradients from the CNS to plasma [21].

In the event of CNS injury such as stroke, cell membrane depolarization from ATP breakdown increases the release of glutamate, while also blocking reuptake of the neurotransmitter due to the consumption of the energy source [22]. The massive release of glutamate overwhelms regulating mechanisms, leading to a build-up of the neurotransmitter in the extracellular milieu. In turn, the excess glutamate activates a series of downstream mediators in the affected tissue which ultimately leads to neuro-excitotoxicity. Cellular death causes more increase in extracellular glutamate, which feeds into the cycle of further cellular death [23] (Fig. 1).

Ionotropic glutamate receptors include the NMDA, AMPA, and kainate types. The major receptor involving glutamate-mediated neuronal damage is the NMDA receptor (NMDAR), an important tri-subunit receptor essential to neuronal plasticity (i.e. learning and memory formation) [6]. Overactivation of extrasynaptic NMDARs, in particular, may be the chief culprits, whereas the activation of synaptic NMDARs have been shown to actually confer neuroprotection. The state of neuronal health is thus dependent on the delicate balance between the activation of synaptic versus extrasynaptic receptors [24]. Studies have shown that increased activation of the latter by high levels of glutamate plays a significant role in neuronal excitotoxicity by receptor-mediated influx of calcium [25]. The increased intracellular calcium may then lead to the activation of other mechanisms, including NOS and mitochondrial toxicity [26–28] (Fig. 2).

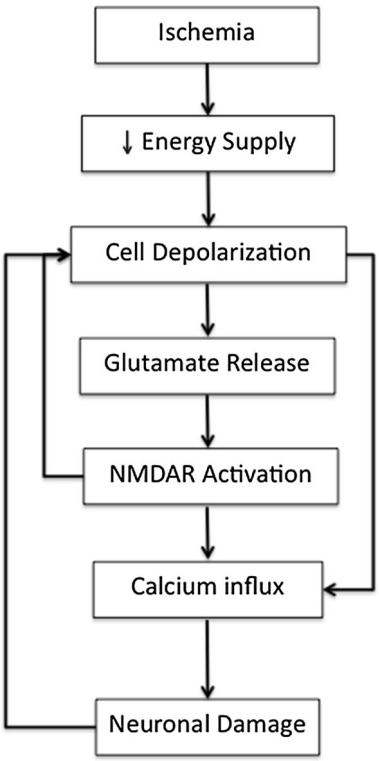

Fig. 1 Downstream effect of neuronal insults leading to activation of NMDARs by glutamate and calcium influx, ultimately resulting in neuronal damage. *NMDAR* NMDA receptor

Nitric oxide production plays a significant role in glutamate-mediated neuronal damage. Neuron injuries such as ischemia have been shown to induce translocation of neuronal NOS (nNOS) from the cytosol to the cell membrane where it can interact with NMDARs [29]. Studies have demonstrated that NMDARs are spatially linked with NOS via the postsynaptic density protein of 95 kDa (PSD-95) [30–32]. During glutamate binding to NMDARs, the influx of calcium leads to the activation of the nearby NOS, resulting in the production of NO [15, 33]. NO can in turn lead to formation of harmful oxidants, causing protein nitration, protein oxidation, lipid peroxidation, direct DNA damage, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) depletion [29, 32].

Neuron degeneration can also arise from the formation of free radicals via damage of mitochondria after massive NMDAR-mediated glutamate insult. Again, increase in calcium is implicated [15, 34]. A study by Dykens demonstrated that the increase in calcium concentrations after NMDA activation leads to an increase in the mitochondria sequestration through high capacity sodium/calcium exchangers [35]. However, the elevated utilization of these exchanges can result in metabolic acidosis, as well as the activation of superoxide and other free radical production. Mitochondrial injury also initiates calpain cleavage of key regulatory proteins and activation of pro-apoptotic genes, leading to cell death [23].

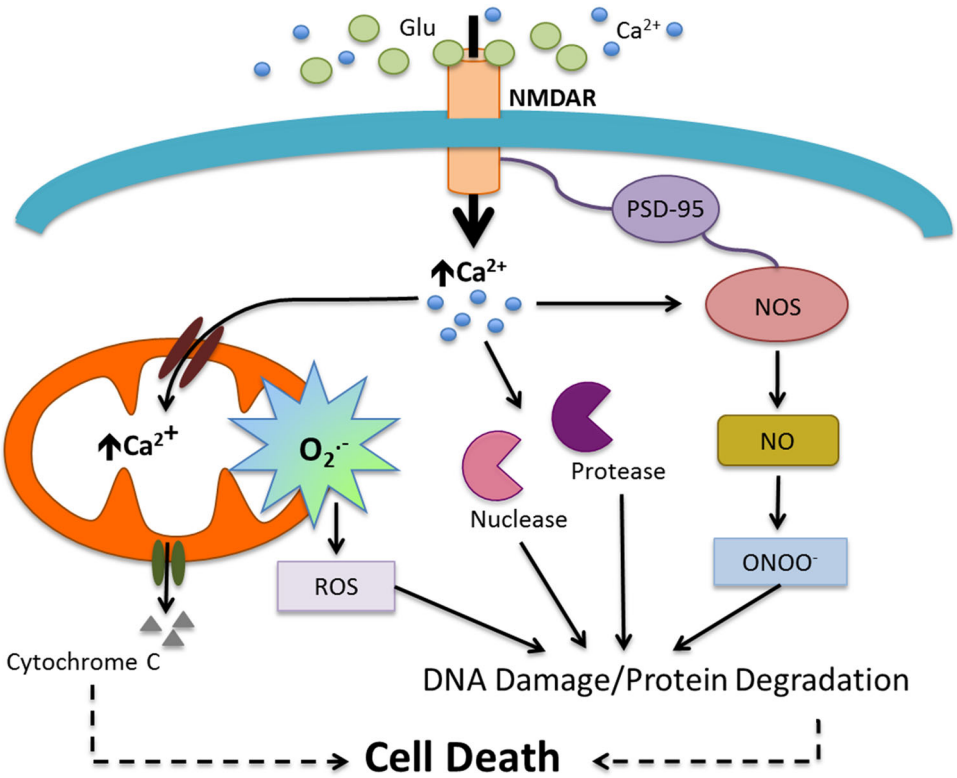

Fig. 2 Effects of massive calcium influx leading to ROS formation, DNA/protein degradation, and activation of cell death pathways. *ROS* reactive oxygen species, *Glu* glutamate, *NMDAR* NMDA receptor, *PSD-95* postsynaptic density protein of 95 kDa, *NOS* nitric oxide synthase, *NO* nitric oxide, *ONOO*$^{-}$ peroxynitrite

3 Past Endeavors at Curbing Excitotoxicity

Elucidation of the mechanisms behind glutamate excitotoxicity ushered in a wave of pharmacological advances aimed at exploiting this newfound knowledge. In the beginning, the major focus of research centered on NMDAR antagonism. NMDARs provided a logical target for drug design as they represented a major gateway for the myriad of other downstream effects of glutamate excitotoxicity. Moreover, during this period, progress in protein biochemistry and small molecule design yielded a wealth of information regarding the structure and function of these receptors [36].

Several classes of NMDAR antagonists with different sites of action were developed, namely the competitive NMDAR antagonists acting on glutamate or glycine binding sites, non-competitive allosteric inhibitors acting at other extracellular sites, and NMDAR channel blockers, which acted on sites in the receptor channel pore [37]. Although showing promise in animal studies, antagonist drugs such as selfotel, gavestinel, and traxoprodil have largely failed in randomized controlled clinical trials in humans. A variety of reasons have been postulated in explaining the lack of success for these NMDAR-targeting therapies. Many of these compounds lack sufficient brain penetrance while exhibiting significant dose-limiting side effects [37]. The adverse events profile included hallucinations, agitations, catatonia, peripheral sensory loss, nausea, and elevation in blood pressure [7]. Moreover, in the setting of acute CNS insults, such as strokes or traumatic brain injuries, glutamate excitotoxicity is thought to cause harm within a narrow timeframe, after which the neurotransmitter reassumes its normal function. Therefore, the use of agents acting on NMDARs, a major receptor of glutamate, may have not only missed the window for therapeutic efficacy but also led to undesired side effects from prolonged receptor blockade [12].

Research has continued on NMDAR antagonism despite initial disappointments. Recent studies have suggested that a possible solution may lay in distinguishing between NMDARs located within and outside of the synapse as they have virtually opposite downstream effects [24]. Memantine, a non-competitive NMDAR antagonist currently used as a treatment for AD provides a successful example of this concept [38]. AD is a chronic CNS disease with a pathogenesis that involves NMDAR-dependent binding and accumulation of amyloid-β (Aβ) oligomers in affected brain tissue [39]. In turn, these Aβ oligomers induce reactive oxygen species (ROS) formation, leading to oxidative stress and cell death, via pathways requiring NMDAR activation [40]. The drug memantine has been shown to disrupt these pathogenic processes by selectively blocking the tonically activated extrasynaptic NMDARs while leaving synaptic NMDARs largely undisturbed.

The increase in understanding of mechanisms governing NMDAR activation and its downstream effects have thus led to renewed interest in exploring the possibilities of therapeutics against NMDARs. On the other hand, studies in the last two decades have expanded beyond the NMDAR with newer experiments seeking ways to control the upstream glutamate concentration as well as downstream protein signals. These newer treatments have also shown much promise in curbing the deleterious effects of glutamate excitotoxicity. Some of the potential therapies to target glutamate excitotoxicity are described in Table 1.

4 Beyond NMDA Receptor Antagonism: Potential Agents Against Glutamate Excitotoxicity

4.1 Blood-to-Brain Glutamate Scavenging

Blood-to-brain homeostasis of glutamate is mediated by several glutamate transporters. Danbolt suggested that high glutamate concentration at the synaptic cleft are rapidly (up to 1,000-fold) reduced by the action of glutamate transporters present on both nerve terminals and surrounding astrocytes to prevent glutamate excitotoxicity [5]. There is an unfavorable gradient between blood (40–60 μM) and brain (1–10 μM) glutamate concentrations. Na^+-dependent transporters (EAAT3) on the antiluminal membrane act to accumulate the excess extracellular glutamate into the endothelial cells [21], and when endothelial glutamate concentration becomes higher than the blood glutamate concentration, glutamate is transported into the blood by means of facilitated diffusion. A mechanism that facilitates blood excretion of glutamate is its conversion into α-ketoglutarate. The organic compounds pyruvate and oxaloacetate are two of the co-substrates that can be used. Pyruvate, in the presence of glutamate pyruvate transaminase (GPT) can convert glutamate into α-ketoglutarate. Similarly, oxaloacetate, in the presence of glutamate oxaloacetate (GOT) transaminase, can convert glutamate into α-ketoglutarate.

Based on the brain-to-blood transport of glutamate, studies have demonstrated that increasing the glutamate concentration gradient between the brain and the blood using blood glutamate scavengers (oxaloacetate, pyruvate, GPT, and GOT) could potentiate the efflux of glutamate from the brain [1, 41]. Peripheral injection of GOT and GPT in rats, whether alone or in combination with oxaloacetate or pyruvate, respectively, led to a significant reduction in blood glutamate levels [28, 42, 43]. Because glutamate receptor blockage interferes with normal cellular signaling, it is understandable that related therapeutic routes have thus far been deceiving. On the other hand, glutamate scavengers are thought to only affect glutamate

Table 1 Therapies against glutamate excitotoxicity

Category	Compound name	Mechanism of action	Neurological disease	Status
Glutamate scavenging	Oxalocetate	Increase brain to blood Glu gradient via promoting conversion of glutamate into α-ketoglutarate [28, 42, 43]	Ischemic stroke, subarachnoid hemorrhage, traumatic brain injury [26, 42, 47, 49]	Animal studies
	Pyruvate	See oxalocetate	See oxalocetate	Animal studies
Selective NOS inhibition	3-bromo-7-nitroindazole (3-BNI)	Downregulation of downstream endoplasmic reticulum stress and pro-apoptotic pathways [57]	Diabetic stroke [57]	Animal studies
	S-nitrosoglutathione (GSNO)	Blocking of S-nitrosylation of Fas [58]	Global ischemic injuries [58]	Animal studies
	S-methyl-L-thiocitrulline (SMTC)	Inhibition of matrix metalloproteinases, decreasing iron and bilirubin toxicity [59]	Intracerebral hemorrhage [59]	Animal studies
PSD-95 disruption	Tat-NR2B9c peptide	Disruption of NMDAR to PSD-95 interactions [30]	Ischemic stroke [30]	Animal studies
	ZL006	Prevention of NMDAR and NOS interaction via dissociation of nNOS from PSD-95 [31, 32, 61, 62]	Ischemic stroke [31, 32, 61, 62]	Positive results in primates. Awaiting human trials [61, 62]
Free radical scavenging	Edaravone	Suppression of PERK/eIF2α/ATF4 pathways [64]	Ischemic stroke [63–66]	Phase III [66, 67]
	Ebselen	Decrease of ROS reduction reactions via effects on GABA shunt enzymes [76–79]	Ischemic stroke [80, 81]	Phase III [80, 81]
	Curcumin	Suppression of lipid peroxidation, inhibition of NF-κB pathway and preservation of GLT-1 expression [84]	Subarachoinoid hemorrhage, focal cerebral ischemia, intracerebral hemorrhage [84–86]	Animal studies

Glu glutamate, *NOS* nitric oxide synthase, *PSD-95* postsynaptic density protein of 95 kDa, *PERK* protein kinase R-like endoplasmic reticulum kinase, *eIF2α* α-subunit of eukaryotic initiation factor 2, *ATF4* activating transcription factor 4, *ROS* reactive oxygen species, *GABA* γ-aminobutyric acid, *NF-κB* nuclear factor κB, *GLT-1* glutamate transporter type 1

concentration and do so in ways that only involve physiological reduction of glutamate in areas of the brain where levels are thought to be pathologic [41]. It was also demonstrated that the process of scavenging dies down as brain glutamate concentration begins to approach physiological levels [43]. For these reasons, clinical studies have trended towards the exploration of novel therapeutic solutions involving blood glutamate scavengers.

Both pyruvate and oxaloacetate demonstrated neuroprotective benefits in rats with traumatic brain insults [17, 28, 42, 43] where increases in brain extracellular glutamate concentrations occurred [44, 45]. Administration of blood glutamate scavenger pyruvate and oxaloacetate before TBI infliction, and 30 and 60 min after TBI were all shown to be effective [42]. Experiments have demonstrated that the benefits of oxaloacetate were more likely to be from its scavenging properties than from a different mechanism. One instance was the use of malate, a GOT blocker, to demonstrate abolishment of oxaloacetate-induced improvement of neurological severity score and oxaloacetate-induced reduction of blood glutamate concentration in closed head injuries [43]. Another example was the reduced neuroprotective benefit of oxaloacetate (1 mmol per 100 g of rat weight) observed with simultaneous injection of oxaloacetate and glutamate in rats with TBI, suggesting the neutralization of oxaloacetate-induced decreases in blood glutamate levels as the principal cause [42].

Other than TBI, the use of glutamate scavengers has also been explored in stroke, subarachnoid hemorrhage (SAH), epilepsy, migraine, and other central nervous system (CNS)-related insults. In human stroke studies, increased glutamate concentration in the blood and cerebrospinal fluid (CSF) has been associated with poor outcome [16, 46], while increased concentration of blood GOT and GPT have been associated with good outcomes [47]. In rats with photothrombic-induced lesions or incomplete forebrain-induced ischemia, peripheral oxaloacetate administration led to reduction in infarct size [48]. Peripheral infusion of oxaloacetate with or without GOT [47, 49] and pyruvate (with or without GPT) [50] within 60 min of middle cerebral artery (MCA) occlusion also resulted in reduction in infarct size and reduced brain edema in rats. Recently, a study by Knapp et al. [51] on focal ischemic models in rats further demonstrated that after an ischemic event, animals treated with oxaloacetate regained significantly more neurologic function when compared with controls.

In the study by Boyko et al. [26], initiation of pyruvate or oxaloacetate 60 min after SAH induction showed a

significant decrease in CSF glutamate concentration, a decrease in the breakdown of the blood–brain barrier, and an improvement in neurological severity score when compared with placebo (infusion of normal saline). Another study showed that injection of a single dose of oxaloacetate and pyruvate 30 min following pilocarpine-induced status epilepticus led to a reduction in hippocampal neuronal loss [52], suggesting glutamate scavengers as a target for novel epileptic therapy. Campos et al. demonstrated that patients with migraine had significantly lower peripheral GOT activity compared with controls. With excess glutamate playing an important role in organophosphate-induced seizures [27, 53], it was shown that blood glutamate scavenging using a combination of oxaloacetate and/or human recombinant GOT was neuroprotective treatment in paraoxon toxicity in rats (an organophosphate) [54].

However, the means of reducing glutamate concentration is not solely relegated to pharmacological strategies. Most recently, researchers have been examining ways to directly remove glutamate from the body, namely via peritoneal dialysis (PD). In patients undergoing PD, serum glutamate levels have been shown to decrease as much as 20 % within 1 h of the procedure [55]. Applying this concept to rat models subjected to MCA occlusion, Godino et al. [56] showed that PD significantly attenuated the rise in serum glutamate after the insult, and in turn successfully reduced infarct size. Furthermore, their study demonstrated, using functional magnetic resonance imaging, that the preserved tissue retained functional capacity. It has been postulated that such extracorporeal methods of glutamate reduction are advantageous compared with pharmacological therapies such as oxaloacetate and pyruvate as they convey even less side effects. However, as with the glutamate scavengers, much work needs to be undertaken to assess the safety and efficacy profiles of PD in excitotoxic brain injuries before human trials are possible.

Overall, the strategy of blood-to-brain glutamate reduction has shown great promise as future novel therapy. Glutamate serves as a good target as it represents the beginning of the ischemic cascade (Fig. 1). Furthermore, unlike glutamate receptor modulators, reduction of blood glutamate levels is thought to interfere less with intracellular signaling and thus have better side effect profiles. On the other hand, one perceived limitation faced by glutamate scavenging is a narrow therapeutic window, in particular, during acute neurological injuries such as ischemia. For example, therapies that reduce glutamate must, in theory, be rendered almost immediately to a stroke patient as glutamate levels increase and exert its effects almost immediately after an ischemic event. With this in mind, the next two sections discuss potential targets more downstream of glutamate. These methods, although not as elegant as glutamate scavenging, offer their own advantages, including a higher therapeutic window during which to implement treatment.

4.2 Nitric Oxide Synthase Inhibition

The activation of NOS represents one of the first steps in NMDA-mediated excitotoxicity. Not surprisingly, there have been many attempts to target NOS in an effort to convey neuroprotection after glutamate insult. As described by Lau and Tymianski, initial efforts using the molecule L-NG-nitroarginine methyl ester (L-NAME) to suppress NOS activity through non-specific inhibition yielded mixed results due to the contrasting properties of synthase isoforms [25]. Later studies showed the nNOS to be the major culprit involved in CNS injuries and degeneration, leading to the development of more specific nNOS inhibitors such as 3-bromo-7-nitroindazole (3-BNI) and *S*-nitrosoglutathione (GSNO) [57, 58]. 3-BNI has been shown to confer neuroprotection in rat models of ischemic injury from diabetic stroke. The proposed mechanism involves inhibition of downstream endoplasmic reticulum stress pathways and pro-apoptotic transcription factor CCAAT/enhancer binding protein (CHOP) [57]. GSNO, like 3-BNI, has also been shown to protect against global ischemic injuries in rats, but through *S*-nitrosylation and inactivation of nNOS, which, in turn, blocks the *S*-nitrosylation of Fas and its associated apoptotic pathway [58].

More recently, research from Lu et al. demonstrated that the inhibitor S-methyl-L-thiocitrulline (SMTC) can attenuate intracerebral hemorrhage (ICH)-induced neuronal cell death and improve functional recovery in rats. The study showed that inhibiting nNOS prevented increase in matrix metalloproteinase activation, leading to better outcomes. Furthermore, nNOS inhibition was effective in decreasing the toxicities precipitated by the increase of iron and bilirubin, both of which are elevated in the tissue environment of ICH injuries [59].

Another method related to suppressing nNOS activity involves the disruption of the NMDAR to PSD-95. As described above, PSD-95 is a protein that spatially links the NMDAR with nNOS and plays a critical role in activation of the latter after calcium influx through the NMDAR [30–32]. Aarts et al. demonstrated that the protein–protein interaction between PSD-95 with NMDAR and nNOS represented a new therapeutic target against excitatory neuron damage. The molecule they used, an exogenous peptide mimicking the carboxyl-terminal of an NMDAR subunit, barred the receptor from binding with PSD-95 and led to neuroprotection in ischemic brain injuries. Importantly, because of the specificity in the targeting of PSD-95, NMDAR activity is not affected, leading to better side effect profiles [30].

A subsequent study by Zhou et al. produced a similar drug, ZL006, capable of dissociating nNOS from PSD-95 and preventing its translocation to the cell membrane where it interacts with NMDARs. ZL006 had potent neuroprotective activity in animal studies, shown to reduce infarct size when administered up to 3 h after ischemia [32]. The molecule also had minimal effects on NMDAR function and was further established to exert no effects on nNOS catalytic activity, thereby avoiding adverse symptoms of aggression, slowed learning, and memory loss. Initial studies on non-human primates by the Tymianski group have shown promising neuroprotection, as evidenced by reduction in infarct volumes, preservation of ischemic cells' transcriptional capacity, and enhancement of behavioral functions after ischemic stroke [60]. Efforts are currently being made to translate these positive results to human trials [61].

4.3 Free Radical Scavenging

Free radical scavengers provide a means of stemming the adverse consequences of glutamate excitotoxicity further downstream of NMDARs and nNOS. Thus far, there have been many agents that have shown varying degrees of neuroprotection after glutamate insult. However, most of the positive results have largely been confined to animal experiments. Outcomes in human studies have been mixed, with several drugs, such as NXY-059, not being able to translate their success in clinical trials [62]. Despite setbacks, several potential therapies targeting free radicals and ROS are currently being investigated.

4.3.1 Edaravone

Edaravone is a low molecular weight hydroxyl free radical scavenger that has been approved in Japan for the treatment of acute ischemic stroke within 24 h after onset [63]. The antioxidant effect of the compound is thought to be from the suppression of the PERK/eIF2α/ATF4 integrated response pathway that ultimately activates caspase-12 for apoptosis [64]. Results on edaravone have ranged from modest to drastic improvements in the almost 20 years of clinical experience [63, 65]. As per the Cochrane review performed by Yang et al., there are three completed randomized control trials involving edaravone, not used in combination with another agent, in acute ischemic stroke [66]. Of these, only the Otomo trial could be verified on the PubMed database. This trial, a randomized, placebo-controlled, double-blinded, multicenter study showed a significant difference between the edaravone-treated and placebo groups using the modified Rankin scale scoring system ($p = 0.0382$) at time intervals to treatment of less than 24, 25–48, and 48–72 h. According to the study, the most pronounced improvement occurred when the drug was administered within a day after thrombotic events [67].

Subsequent retrospective reports, such as the Mishina et al. and Ohta et al. studies, demonstrated that the protective effects of edaravone also extended to lacunar infarcts, although only in milder cases and to a limited extent (i.e. no difference in the National Institutes of Health Stroke Scale scores between groups but a larger decrease in palsy score in the edaravone-treated group) [68–70]. Moreover, the drug has relatively mild adverse side effects, although renal and hepatic toxicity have been reported [71, 72]. Currently, the combined use of edaravone with tissue plasminogen activator (tPA) in thrombotic stroke is being explored, with early preclinical experiments showing that the combined therapy exhibits synergistic benefits [73].

4.3.2 Ebselen

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is an organoselenium therapeutic that catalyzes reduction reactions of ROS responsible for lipid peroxidation, protein oxidation, and DNA damage, much like that of glutathione peroxidase [74, 75]. Various animal studies have shown that ebselen protects against ischemic and reperfusion damage in both gray and white matters of the brain, likely through the control of expression of gamma-aminobutyric acid (GABA) shunt enzymes that supply the tricarboxylic acid (TCA) cycle [76–79]. Upon searching the PubMed database, as well as the Stroke Trials Registry, two published placebo-controlled, double-blind trials involving ischemic strokes were found. The study by Yamaguchi et al. [80] demonstrated improved clinical outcome, as measured by the Glasgow Outcome Scale and modified Mathew Scale, in patients treated with ebselen versus placebo when the agent was given within 24 h after the onset of stroke. Similarly, Ogawa et al. [81] conducted a trial in patients with complete occlusion of the MCA, showing that there was significant reduction in volume of cerebral infarct and patient outcome if treatment was begun within 6 h of disease onset. Ebselen was never approved for public use due to borderline efficacy in phase III trials [74]. Despite this, research continues on the compound and has diversified to other neurodegenerative pathologies such as traumatic brain injuries [82, 83].

4.3.3 Curcumin

Diferuloylmethane, better known as curcumin, is an antioxidant with known properties to scavenge free radicals and suppress lipid peroxidation [84]. Kuo et al. showed that the compound can considerably lower glutamate levels in rat models of SAH, resulting in significantly higher

neurologic scores and lower mortality compared with positive controls. In the study, curcumin was shown to preserve glutamate transporter GLT-1 expression and function, leading to adequate removal of glutamate and attenuation of excitotoxicity [85]. Subsequent studies have also shown that the molecule confers neuroprotection in animal models of focal cerebral ischemia and ICH, possibly through the inhibition of the nuclear factor (NF)-kB signaling pathway [86]. Currently, there are no human trials involving curcumin in acute or chronic neurodegenerative disease.

4.3.4 Other Free Radical Scavengers

Many other free radical scavengers and antioxidants are currently under investigation. Some of these include ferulic acid, a phenolic phytochemical, ascorbate, and α-phenyl-*N*-tert-butyl-nitrone, a spin-trap scavenger also known as PBN [87–89]. Again, although the initial animal studies for these agents have shown potential, human trials need to be performed to assess for any implications in the clinical setting.

5 Conclusions

In recent years, research on glutamate excitotoxicity has generated considerable data on the molecular causes underlying neuronal injury from stroke, cerebral trauma, and SAHs, among other acute neurological pathologies. With a better understanding of the mechanisms by which elevated glutamate ultimately leads to cell death, scientists are devising novel therapeutic agents that target different steps in the pathway. In this article, we sought to describe major strategies that are in development, namely glutamate scavenging, which directly lowers extracellular glutamate in the CNS, as well as nitric oxide inhibitors and free radical scavengers, which exert neuroprotection downstream of glutamate activation of NMDARs. Glutamate scavenging, in particular, has gained much attention as a novel approach in stemming excitotoxicity. As these different methods aim to affect the glutamate-mediated insult at different levels, it may be interesting in the future to investigate the use of drug combinations to look for synergy. Most importantly, further studies involving randomized clinical trials in humans are needed to determine whether these agents, successful in animal models, can indeed translate into drugs with true clinical value.

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