Comprehensive Insight of Neurodegenerative Diseases and The Role of Neurotoxin Agents — Glutamate

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Abstract: The increased concentration of extracellular glutamate has been reported to play a key role in most of the neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, even though its importance as an amino acid neurotransmitter in mammalian. Glutamate toxicity, which can be caused by excessive intake of monosodium glutamate (MSG), is the major contributor to pathological neuronal cell death. It causes neuronal dysfunction and degeneration in the central nervous system (CNS). Glutamate neurotoxicity can be categorized into two forms, which are receptor-mediated glutamate excitotoxicity and non-receptor mediated glutamate oxidative toxicity. The receptor-mediated glutamate excitotoxicity involved excessive stimulation of glutamate receptors (GluRs) which lead to excessive ion calcium (Ca^{2+}) influx and activates a cell death cascade involving the accumulation of mitochondrially generated reactive oxygen species (ROS). Studies showed excessive extracellular glutamate leads to nerve cell death via the activation of *N*-methyl-D-aspartate (NMDA) receptors in the cases of trauma or stroke. Whereas non-receptor mediated oxidative toxicity involved the breakdown of the cystine/glutamate antiporter (x_c^-) mechanism, which leads to the depletion of glutathione (GSH) and causes oxidative stress and cell death. The cystine/glutamate antiporter couples the import of cystine to the export of glutamate. The increased concentration of extracellular glutamate could inhibit the uptake of cystine, which is required for the synthesis of the intracellular antioxidant GSH. GSH plays an important role in the disposal of peroxides by brain cells and in the protection against ROS. Depletion of GSH renders the cell to oxidative stress and ultimately leading to cell death. This article aims to provide a comprehensive review of neurodegenerative diseases and the role of neurotoxin agents, glutamate in these diseases.

Keywords: neurodegenerative diseases; Alzheimer's disease; Parkinson's disease; neurotoxin agents; glutamate

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INTRODUCTION

Nervous System

Nervous system is the major controlling, regulatory and communicating system in the body responsible for receiving and transmitting stimuli throughout the body[1]. Nervous system also monitors and coordinates internal organ function and initiates response to the changes in the external environment. Nervous system can be divided into central nervous system (CNS) and peripheral nervous system (PNS). CNS is composed of the brain and spinal cord, which serves as the main processing center for the entire nervous system[2]. It receives information from and sends information to the PNS. PNS is made up of all the nerves connecting the body to the brain and spinal cord. Sensory neuron is responsible in sending information from internal organ or from external stimuli to CNS, whereas motor neuron transmits information from CNS to organs, muscles and glands[2]. The motor nervous system can be further divided into two systems; autonomic nervous system and somatic nervous system. Autonomic nervous system is responsible for involuntary or visceral bodily function while somatic nervous system is mediating voluntary reflexes for instance skeletal muscle. In general, activity of the nervous system depends on the connectivity among two cell types, which are neurons and glia[3–5].

Neuronal Cell

Neuronal cell is an excitable nerve cell that processes and transmits information by electrochemical signaling. Most neurons cannot divide; thus, neurons cannot be replaced if lost due to injury or disease[1]. There are 3 types of neurons based on functions, which are sensory neurons, motor neurons, and interneuron. Interneurons carry information between sensory neurons and motor neurons and can be found in CNS.

Generally, neuronal cell is comprised of neuronal cell body (soma), axon and dendrite. The axon has a terminal end that constitutes the pre-synaptic site of the synapse while the dendrite has an apical site and multiple branches. Neuron communicates with each other at synaptic cleft (synapse), which involved release of neurotransmitter by pre-synaptic cell and binding of neurotransmitter to the receptor on post-synaptic cell. Neurotransmitter will trigger an electrical response or a secondary messenger pathway that may either excite or inhibit postsynaptic neuron[4]. Excitatory neurotransmitter causes changes that generate action potential in the responding neuron while inhibitory neurotransmitter will block the changes[6,7]. Major neurotransmitters found in the body include glutamate, dopamine, glycine, gamma-aminobutyric acid (GABA) and serotonin.

Glial Cell

The glial cells provide vital support for the proper functioning of neurons. Glial cells are also known as nerve glue, are non-neuronal cells of the nervous system. It does not transmit electrical impulses, but it appears to be the most abundant cell types in the CNS. Glial cell can be divided into macroglia (astrocytes, oligodendroglia and Schwann cells) and microglia. Different types of glial cell provide different support to neurons. Microglia cells in CNS protect neurons from bacteria invasion. Oligodendrocytes and Schwann cells provide myelination to axons in CNS and PNS respectively. Myelin sheath provides insulation to the axon that allows electrical signals to be transmitted efficiently[8]. Astrocytes not only provide physical support to neurons, but also support neurons by providing antioxidant protection, substrates for neuronal metabolism via neurovascular coupling, digest dead neurons and glutamate clearances[4,9]. Also, astrocytes have interaction with the cerebral endothelium in determining blood brain barrier function, morphology and protein expression[10].

Neuron-astrocyte interaction in the brain

Astrocytes are the most numerous and diverse glial cells in the CNS. Many studies demonstrating astrocyte dysfunction throughout the neurodegenerative process as a prominent determinant for survival for both neuronal cell and the entire organism. Neuronal activity leads to increase of potassium ion (K^+) and glutamate levels in extracellular space. As a supportive cell, astrocyte plays important role in maintaining the extracellular K^+ homeostasis through potassium channels expressed by astrocytes[11]. Moreover, astrocytes are known to express high level of glutamate transporter to remove glutamate from extracellular space[12]. The increased concentration of extracellular K^+ and glutamate will trigger astrocyte glycolysis and further enhance lactate and pyruvate production to fuel neuronal tricarboxylic acid cycle (TCA cycle)[13,14]. Astrocyte and neuronal cell also coupled by glutamate-glutamine cycle. Astrocytes take up glutamate and convert it to glutamine. This glutamine is then shuttled back to presynaptic terminals (neuronal cells) and used by neuronal cell to replenish the neurotransmitter glutamate[15]. In addition, neurotransmitters released by neuronal cells induce transient elevations of internal Ca^{2+} levels in astrocytes due to the activation of metabotropic glutamate receptors on astrocytes[16,17]. The increased level of astrocytic Ca^{2+} triggers the release of chemical transmitters from astrocytes, which can cause sustained modulatory actions on neighbouring neuronal cells[17].

NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are becoming huge public health challenge due to their increasing medical and social impact. It is one of the major global health burdens in the Western world and is often found in aging population[18]. These diseases can be life-threatening and have great impacts on both the patients and caregivers. Neurodegenerative diseases can be illustrated by progressive nervous system dysfunction that causes deterioration of many of human body's activities, including speech, movement, balance, heart function and breathing[19]. The causes of neurodegenerative diseases comprise genetic factors, infections or autoimmune diseases, brain trauma or injury and environmental toxins[20–23]. Most of the neurodegenerative disorders are incurable; thus, treatments are applied with the hope to relieve pain, improve symptoms, and increase mobility. Neurodegenerative diseases are categorized based on primary brain region or type of affected brain cell, clinical symptoms, and type of protein aggregated in brain[24]. Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) are the most common age-related neurodegenerative diseases. Some forms of these diseases could be inherited. Moreover, morphological, biochemical, genetic, cell and animal model studies reveal that mitochondria play roles in the neurodegeneration through rendering vulnerable neurons susceptible to stress, cellular aging and genetic variations[25].

Alzheimer's Disease (AD)

Alzheimer's disease (AD) can be categorized by progressive degenerative disorders of the brain which causes irreversible loss of neurons and memory. It is one of the common causes leading to dementia. Late-onset of Alzheimer's disease (LOAD) with age 65 years or older is representing the majority of AD. Senile plaques, intracellular neurofibrillary tangles (NFTs), extracellular degenerating neurons, dystrophic neuritis, and activated

astrocytes or microglial found in the brain underlie the pathogenesis of AD[26]. Senile plaque is mainly composed of β-amyloid (Aβ) while NFTs consist of hyperphosphorylated and aggregated forms of the tau protein. Tau is a microtubule-binding protein that acts as neuronal cytoskeleton stabilizer and participates in vesicular transport and axonal polarity. The deposition of hyperphosphorylated tau protein impairs axonal transport therefore affecting the nutrition of dendrites and axon terminals. While the accumulation of Aβ activates cellular stress responses and disrupts signal transduction pathways[27]. Studies indicated that Aβ also induces inflammatory reaction in the brain and causes release of various cytokine such as TNF-α (tumor necrosis factor-alpha)[28]. TNF-α could induce infiltration of leukocytes and possible T-cells into the central nervous system through its capacity in enhancing blood brain barrier permeability[29]. The increased permeability of blood brain barrier causes influx of neurotoxin, such as glutamate and arachidonic acid, which could lead to substantial damage to the brain. Cholinergic and glutamatergic neurotransmission systems, which play role in cognition, were affecting in patients with Alzheimer's disease[30].

Parkinson's Disease (PD)

Parkinson's disease (PD) is the second most common late-life neurodegenerative disease after Alzheimer's disease. It can be characterized by progressive dopaminergic cell loss in the substantia nigra pars compacta which leads to dysfunction of the basal ganglia system and motor symptoms and subsequently causing tremors, slow movement, rigidity and gait impairment[31,32]. Another pathological feature of PD is the accumulation of Lewy bodies in neurons of the brainstem nuclei, substantia nigra, hippocampus, cerebral cortex, myenteric plexus, and olfactory bulb. Lewy bodies are accumulations of microscopic protein deposits in the brain, mainly alpha-synuclein. The pathology of PD is not yet fully understood. Some genetic and toxin-based animal models suggest that inflammation, oxidative stress, mitochondrial dysfunction, aberrant processing of proteins by the ubiquitin-proteasome system, and activation of apoptotic pathways are playing roles in dopaminergic cell death[33]. Furthermore, some studies indicated the increased glutamatergic transmission may contribute an excitotoxic component to the cellular insults that lead to degeneration in the substantia nigra pars compacta as well as the glial response that arises in the striatum and the substantia nigra[34].

Other Neurodegenerative Diseases

Other neurodegenerative disorders include Huntington's disease (HD) and amyotropic lateral sclerosis (ALS). HD can be categorized by cognitive decline, psychiatric disturbances and motor dysfunction, which will lead to dementia and death approximately 15–20 years after the onset of the disease[35]. HD is an autosomal-dominant disorder caused by the expansion of a CAG trinucleotide repeat encoding an abnormally long polyglutamine

track in the N terminus of the huntingtin protein[36]. This protein may inhibit mitochondrial function and proteasome activity[37]. HD has a prevalence of 5–10 cases per 100,000. Thus, HD is known as one the most common inherited neurodegenerative disease[35].

While amyotrophic lateral sclerosis seemed to be a progressive and usually fatal disorder caused by motor-neuron degeneration in the brain and spinal cord. Motor-neuron degeneration in the brain and spinal cord will lead to paralysis of voluntary muscles. The mutation in superoxide dismutase 1 (SOD1) seems to be associated with amyotrophic lateral sclerosis. This mutation will form aggregates in mitochondria which could interfere anti-apoptotic function of B-cell lymphoma 2 (Bcl-2), mitochondrial import and generate free radicals. Reactive oxygen species produced will inhibit the function of glial glutamate transporter (EAAT2) and lead to the increase of extracellular glutamate, next triggering motor neuron death[38].

FACTORS INVOLVED IN NEURODEGENERATIVE DISEASES

Neurodegeneration is due to the death of neuronal cells through the delayed process of apoptosis or necrosis. Among the known risk factors for neurodegenerative diseases, such as genetic polymorphism, infection and head trauma, the role of neurotoxin agents such as glutamate and an oxidative stress received huge attention recently. This is because most of the pathologies of neurodegenerative diseases involve oxidative stress triggered by neurotoxin agents or imbalance in pro-oxidant/antioxidant homeostasis[39,40].

Astrocytes in Neurodegenerative Diseases

Astrocytes have many housekeeping functions, such as the maintenance of the extracellular environment and synaptic function in the CNS. Also, astrocytes play a vital role in the maintenance of neurotransmitter synthesis and neuronal metabolism. Astrocytes become activated (reactive) in response to a variety of brain insults, such as trauma, stroke and neurodegenerative diseases[41]. Reactive gliosis can be characterized by the increase expression of vimentin (Vim), glial fibrillary acidic protein (GFAP), S100β and proliferation that could probable occur in neurodegenerative diseases[42]. Astrocytes will be activated under stress and injury, leading to an upregulation of proinflammatory cytokines and chemokines, which are related to the pathogenesis of AD[43]. Recent studies suggested that activated astrocytes play a role in the clearance of the Aβ-peptide and thus preventing the plaque formation in AD. Furthermore, astrocytosis (abnormal increase number of astrocytes) was discovered in affected brain region of HD patients. The Huntingtin protein was also observed to be co-localized with reactive astrocytes[15].

GLUTAMATE

L-glutamate is a non-essential amino acid which can be gained from alimentary protein, endogenous protein and

also monosodium glutamate (MSG) that is used as food additive[44]. It is one of the most abundant neurotransmitter in mammalian central nervous system and it is involved in numerous normal brain functions, such as memory, cognition and learning[45]. Furthermore, glutamate has an essential role in regulating bio-energetic processes such as reactions of glycolysis, gluconeogenesis, citric acid cycle and synthesis of ketone bodies. It is the precursor for γ-aminobutyric acid (GABA) and glutathione (GSH)[46]. Glutamate is one of the key factors in central pain transduction mechanisms and excitotoxic neuronal cell injury[45].

Glutamate receptors

Neurotransmission occurs at synapses and it involved processes of neurotransmitters carrying signals released from presynaptic neuron terminals to synaptic cleft followed by binding of neurotransmitter to the receptors on the postsynaptic neuron. Binding of excitatory neurotransmitters, such as glutamate, to the receptor proteins on the postsynaptic neuron will cause sodium ion (Na^+) channels to open and allow influx of Na^+ to neuronal cell, and causes the neuron membrane to depolarize due to an action potential. The excitation of glutamate can be mediated via activation of metabotropic glutamate receptors and ionotropic glutamate receptors[47]. There are 3 families of ionotropic glutamate receptors; *N*-Methyl-D-Aspartate (NMDA), α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainate receptors. The ionotropic glutamate receptors are coupled directly to membrane ion channels which conducting only Na^+ or both Na^+ and Ca^{2+}. Metabotropic glutamate receptors comprise of G-protein coupled receptors (mGluR1–8) that are important in regulating ion channels and enzymes producing second messenger[48]. It is categorized into 3 groups based on the sequence, function and pharmacology. Group I mGluRs (mGluR1 and 5) activates phospholipase C via Gq proteins and initiate an inositol triphosphate/diacylglycerol (IP3/DAG) second messenger cascade. Whereas Group II mGluRs (mGluR2 and 3) and Group III mGluRs (mGluR4, 6, 7 and 8) inhibit adenylyl cyclase via $G_{i/o}$ proteins. Reports showed that group II and III were mainly localized on presynaptic neuron while group III was localized on postsynaptic neuron[49]. Metabotropic and Ionotropic glutamate receptors present on both neuronal and glial cells[50,51]. Nevertheless, the involvement of glutamate receptors of glial cells in neurotransmission remains unknown. While the binding of inhibitory neurotransmitters, such as serotonin or GABA, to the receptor proteins on the postsynaptic neuron will lead to hyperpolarization and efflux of potassium ion (K^+) from neuronal cell[52].

Glutamate Transporters

The level of extracellular glutamate must remain low to prevent excessive excitation that can injure or kill neuronal cells due to the deficiency of enzyme at extracellular space to metabolize glutamate. The excessive glutamate can only be removed by cellular uptake through glutamate transporters on the plasma membrane of astrocytes and neuronal cells. Glutamate transporter can be discovered in the plasma membranes and intracellular membrane[53]. Glutamate transporters in the plasma membranes can be categorized into high affinity glutamate transporters and low affinity glutamate transporters. There are 5 high affinity glutamate transporters, also known as sodium-dependent glutamate transporters, have been cloned, namely excitatory-amino acid transporter 1-5 (EAAT1-5). These 5 glutamate transporters catalyze Na^+- and K^+-coupled transport of L-glutamate and also L- and D-aspartate[54]. Each glutamate is co-transported with two or three Na^+ and counter-transport of one K^+[55]. EAAT3, EAAT4 and EAAT5 are known as neuronal glutamate transporters. However, there were studies that showed EAAT4 was expressed in astrocytes as well[56]. EAAT1 and EAAT2, also known as glutamate aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1) respectively, are expressed in astrocytes[57]. These astrocytic glutamate transporters are responsible for the majority of glutamate uptake near excitatory synapse[58,59].

Cystine/glutamate antiporter (system x_c^-) is a low affinity glutamate transporter which also known as sodium-independent glutamate transporter. System x_c^- is composed of two subunits, which are xCT light chain and 4F2 heavy chain. It is also known as cystine transporter which carries cystine into the cell in exchange for internal glutamate by using the transmembrane gradient of glutamate as driving force[54,60]. This system is suggested to mediate the cellular cystine uptake for the synthesis of GSH and maintenance of cystine—cysteine redox balance in the extracellular compartment, which is vital for the cellular protection from oxidative stress and cell death[61].

Glutamate Metabolism

When glutamate taken up by cells, it is not only used as transmitter, but also used for metabolic purposes such as protein synthesis, energy metabolism and ammonia fixation[62]. In the nerve terminal, cytoplasmic glutamate is transported into synaptic vesicles by vesicular glutamate transporters (VGLUTs) and then released to the synaptic cleft by exocytosis. As neurotransmitter, the binding of a transmitter to postsynaptic ionotropic glutamate receptors (AMPA, NMDA and kainate receptors) will cause an ionic influx that depolarizes the neuron (excitation)[63]. Whereas, a slower second messenger-mediated affects occur following the activation of the metabotropic glutamate receptors by glutamate[64]. To avoid excessive excitation that could injure and damage neurons, glutamate in the synapse should be removed rapidly. Glutamate can be removed via the uptake into the postsynaptic compartment, uptake into nonneuronal compartment (astrocyte) and reuptake into the presynaptic compartment. Glutamate uptake into presynaptic and postsynaptic neurons seems to be less important than astrocytic transport due to the membrane potential of astrocytes lower than neurons[65]. In astrocytes, glutamate taken up from extracellular fluid is metabolized by 2 different pathways, which are

tricarboxylic acid cycle (TCA cycle) and glutamate-glutamine cycle. Astrocytes convert glutamate and ammonia to glutamine (non-neuroactive species) via ATP-dependent glutamine synthetase. This pathway further supports the buffering of ammonia, which is a potential neurotoxic species. Glutamine synthetase in the brain is located mostly in astrocytes[66]. Glutamine produced is then released to the extracellular fluid and taken up by neuronal cell. Glutamine taken up into neuronal cell could be hydrolyzed to glutamate and ammonia via the action of phosphate-dependent glutaminase. The regenerated glutamate will be reused as neurotransmitter or potential fuel by being oxidized to α-ketoglutarate for neuronal cells[67,68].

Meanwhile, glutamate taken up by astrocyte could be converted to α-ketoglutarate and ammonia via reversible dehydrogenation by glutamate dehydrogenase (GDH) with either NAD^+ or $NADP^+$ as a cofactor[69]. Alpha-ketoglutarate is metabolized through TCA cycle to succinate, fumarate and malate. Malate is further decarboxylated to pyruvate and reduced to lactate. Both pyruvate and lactate are exported from astrocytes to the extracellular fluid and taken up by neuronal cell. Furthermore, glutamate uptake into astrocytes will stimulate glycolysis, that is glucose utilization and lactate production. Glutamate activates astrocytic Na^+/K^+ ATPase by cotransport Na^+ into the cell via Na^+-dependent glutamate uptake by glutamate transporters. The resulted lactate will provide the fuel for neuronal TCA cycle[70]. Figure 1 denotes the metabolism of glutamate in neuronal cell and astrocyte.
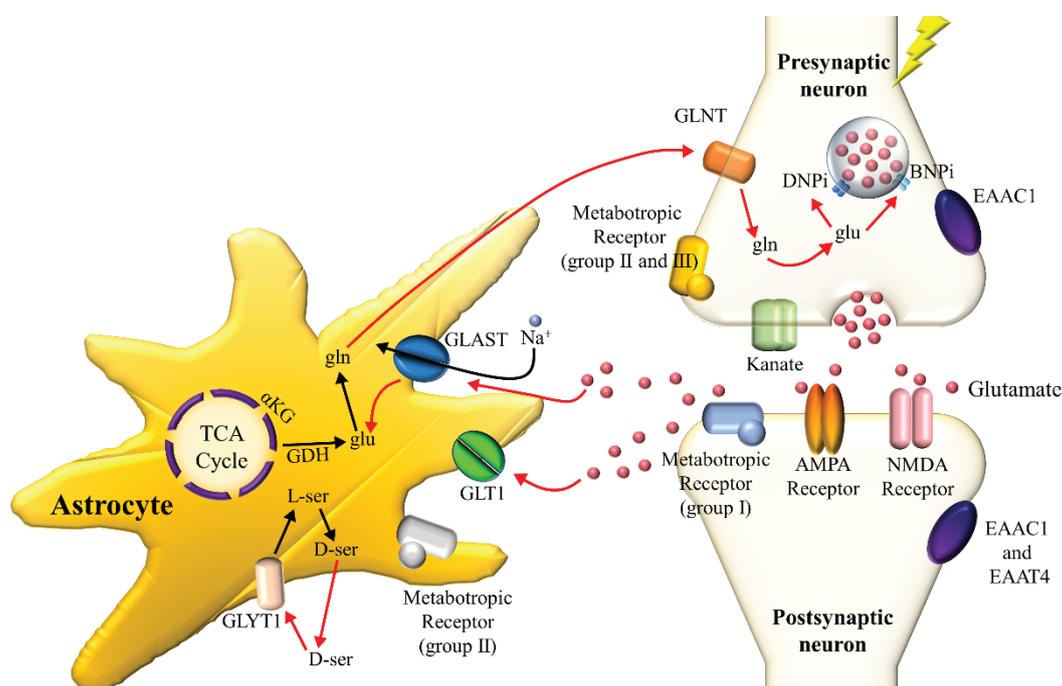

Figure 1. Glutamate metabolism in neuronal cell and astrocyte.

Glutamate Neurotoxicity

The maintenance of a low level (1–3 µM) of extracellular glutamate is vital for normal brain function to prevent excessive stimulation of receptors, excessive formation of ammonia by glutamate dehydrogenase and breakdown of glutamate/cystine antiporter. The excessive accumulation of extracellular glutamate is the main contributor to neuronal cell death under pathological conditions for instance ischemic insults and traumatic brain damage which could be associated to the disruption of glutamate metabolism[71,72]. The disruptions of glutamate metabolism underlie many neurodegenerative pathologies, for instance Alzheimer's disease, Parkinson's disease, Huntington's disease, brain ischemia and amyotrophic lateral sclerosis. These may be associated to variation of glutamate metabolism enzyme activities, disruption in mitochondrial homeostasis, and oxidation or redox imbalance in cells[73,74]. Glutamate neurotoxicity can be classified into receptor-mediated glutamate excitotoxicity and non-receptor mediated oxidative glutamate toxicity.

Receptor-Mediated Glutamate Excitotoxicity

Hayashi was the first to discover the excitatory action of glutamate in 1952 by injecting glutamate into mammalian brain able to produce convulsion[75]. Toxicity of glutamate was then observed when the injection of monosodium glutamate destroyed the neurons in the inner layers of the retina and induced acute neuronal necrosis in brain and term as excitotoxicity[76–78]. Elevated concentration of extracellular glutamate will cause excessive stimulation of glutamate receptors at postsynaptic membrane, which will further induce excessive influx of Ca^{2+} and Na^+. Ionotropic glutamate receptors directly activate channels that allow influx of extracellular Ca^{2+} and Na^+ while metabotropic glutamate receptor stimulates calcium released from internal store (endoplasmic reticulum and mitochondria) [79,80]. The influx of Ca^{2+} and Na^+ through glutamate receptor activation will result in membrane depolarizations which further activate voltage-dependent calcium channels. Plasma membrane calcium transporters regulate the calcium homeostasis by maintaining low concentration of

free intracellular calcium relative to free extracellular. Basically, there are 3 types of membrane transporters, namely antiporters, symporters and ATP-coupled active transporters. Calcium is controlled by the antiporters and Ca^{2+}-ATPase. Antiporters and Ca^{2+}-ATPase move Ca^{2+} out of the neuron and restore Ca^{2+} to mitochondria and endoplasmic reticulum[81]. Antiporter transport Ca^{2+} by a sodium-calcium exchange mechanism while the Ca^{2+}-ATPase transports one Ca^{2+} for each ATP hydrolyzed with a resultant increase in ATP demand. In addition, the direct uptake of Ca^{2+} into the matrix utilizes the proton circuit for oxidation phosphorylation and competes with mitochondrial ATP synthesis[82]. Therefore, mitochondrial ATP synthesis in mitochondria may cease as the Ca^{2+} flood from cytoplasm to the matrix[83]. Moreover, there are studies that showed Ca^{2+} influx from extracellular space activates cytosolic phospholipase A2 and lead to production of arachidonic acid[84,85]. ROS will be created during arachidonic acid oxidative metabolism[86].

Ca^{2+} excess of mitochondrial matrix triggers a mitochondrial permeability transition pore opens in the mitochondrial membrane, which will lead to collapse of mitochondrial membrane potential (MMP) [87]. Furthermore, reactive oxygen species formed from oxidation phosphorylation also contribute to the opening of the mitochondrial permeability transition pore[88]. The maintenance of MMP is vital for ATP synthesis. The collapse of MMP is related to a secondary increase in cytosolic Ca^{2+} concentration and the release of cytochrome *c* followed by caspase activation and apoptosis[89]. Moreover, caspase-independent death can also be induced by MMP through release of caspase-independent death effectors such as apoptosis-inducing factor and endonuclease G[90].

Non-Receptor Mediated Glutamate Oxidative Toxicity

The increased concentration of extracellular glutamate will lead to a prolonged cell death by oxidative stress known as glutamate oxidative toxicity. In glutamate oxidative toxicity, increased concentration of extracellular glutamate interferes with cystine uptake by blocking the glutamate gradient driven cystine/glutamate antiporter (system xc^-)[91]. In facilitating the uptake of cystine, it is vital to exchange with glutamate at an equimolar ratio. The breakdown of system xc^- will lead to the depletion of cystine and glutathione (GSH). In normal situation, imported cystine (oxidized form of 2 cysteines) is reduced to cysteine, which primarily acts as a precursor for GSH synthesis. The depletion of GSH renders the cells to oxidative stress and cell death[92]. Studies indicated that GSH depletion lead to activation of 12-lipoxygenase (12-LOX) and 15-lipoxygenase (15-LOX) and also causes inactivation of GSH peroxidase 4 (Gpx4), which damage mitochondria and resulted to cytochrome *c* and apoptosis-inducing factor (AIF) release, followed by reactive oxygen species accumulation and cell death[93–96].

OXIDATIVE STRESS

Oxidative stress built up caused by an imbalance of free radicals (pro-oxidant) and antioxidants in favor of the former. Free radicals are atoms or groups of atoms that have one or more unpaired electrons which make it extremely reactive[97]. These extremely reactive molecules tend to react quickly with adjacent molecules by donating or capturing an electron in order to gain stability and start the free radical reaction chain. It usually arises during body normal metabolism and increase with age. Also, free radicals can be generated by the immune system to destroy bacteria and viruses during infection. Environmental factors for instance pollution and radiation can further increase the production of free radicals. The most common cellular free radicals include hydrogen peroxide (H_2O_2), hydroxyl ($OH\cdot$), superoxide (O_2^-) and nitric monoxide ($NO\cdot$). High level of free radicals leads to redox imbalance and oxidative damage to biomolecules, such as protein, lipids and DNA, then lead to many chronic diseases in human such as cancer, diabetics, atherosclerosis, post-ischemic perfusion injury, cardiovascular diseases, stroke, chronic inflammation and other degenerative diseases[98].

Reactive oxygen species (ROS) and the reactive nitrogen species (RNS) are extremely reactive among free radicals, which acts as mediators in biological processes for instance respiration, cellular metabolism, gene translation and transcription, neurotransmission and inflammatory-type reactions[99]. Mitochondria are the main contributor to ROS production, and the electron transport chain that is found in the inner mitochondrial membrane uses oxygen to produce adenosine triphosphate (ATP)[100]. Cytochrome oxidase functions as the terminal enzyme of the chain by accepting an electron from cytochrome *c* and transferring it to oxygen (the final electron acceptor in the chain). The reduction of oxygen by one electron at a time by cytochrome oxidase make it becomes highly reactive. Superoxide (O_2^-), the product of one-electron reduction of oxygen, occurs mainly within the mitochondria of a cell[101]. It also functions as the precursor of ROS (such as hydroxyl radical) and as a mediator in oxidative chain reactions[102]. ROS is known to initiate lipid peroxidation by targeting the carbon-carbon double bond of polyunsaturated fatty acids (PUFAs) which are ample in cellular membranes and low-density lipoproteins. High level of oxygen requirement and unsaturated lipids in brain make it particularly susceptible to oxidative damage and lipid peroxidation[103,104]. Neuronal cells are most vulnerable to oxidative damage due to their low antioxidant activity[105]. Furthermore, the metabolism of excitatory amino acids and neurotransmitters in neuronal cells further increase the production of ROS and oxidative stress. Therefore, oxidative stress plays vital role in the pathogenesis of neurodegenerative diseases[106].

MODE OF CELL DEATH

In neuronal cells, calcium ions overload caused by glutamate excitotoxicity activates damaging enzymes for instance phospholipases that produce ROS. ROS induces neuronal injury such as cell membrane disruption, damage to intracellular proteins and DNA, and induction of mitochondrial release of cytochrome *c*, and caspase

activation. The release of cytochrome *c*, caspase activation and DNA fragmentation are the molecular hallmarks of apoptosis[107–110]. Furthermore, cystein protease or calpains are also activated by the elevation of intracellular Ca^{2+}. Calpains cleave various intracellular proteins, such as regulatory protein, membrane channels and cytoskeletal that lead to neuronal cell death by necrosis[111,112].

Apoptosis and necrosis signify 2 significant different cell death mechanisms. Apoptosis, also well-known as programmed cell death or cell suicide, happens under normal physiological conditions. It is an important mechanism in maintaining the homeostasis and regulation of tissue size during postnatal life. Apoptosis is signified by the activation of endogenous endonucleases that are activated directly or indirectly by a cascade of biochemical reactions, which involve cutting of DNA into fragments and consequently causing cell death. Apparently, cells continue to produce proteins and ATP during apoptosis. As a result, each apoptotic body is surrounded by cell membrane which contains intact and functional cell components. Apoptosis causes morphological changes that can be characterized by a deep chromatin condensation and formation of apoptotic bodies, followed by nuclear fragmentation and secondary necrosis that occur at the final stage.

In contrast to apoptosis, necrosis occurs when cells are exposed to extreme variance from physiological condition. It starts with an impairment of the cell's ability to maintain homeostasis. Necrosis can be signified by the loss of metabolic functions and the loss of cell membrane integrity. The cells terminate their proteins and ATP production during necrosis. The morphological changes of necrosis are signified by cell membrane rupture starts with swelling of cytoplasm and mitochondria. Cell membrane ruptures results in the release of the cell's components into the surrounding tissue. This process is recognized as cytolysis. The released cell's components will induce an intense inflammatory response[113–115].

CONCLUSION

The excessive glutamate-induced neurotoxicity had been associated to many chronic neurodegenerative disorders in CNS. Under normal situation, the astrocyte is responsible in clearing up glutamate from synaptic cleft. Hence, minimizes the neuronal injury from glutamate toxicity. However, excessive glutamate will induce oxidative stress in both astrocyte and neuronal cell and lead to cell death. Studies demonstrated that radical scavengers like vitamin E, in the form of tocopherol and tocotrienol, can protect the glutamate-injured cells from oxidative stress. Other than vitamin E, other sources such as microbial resources were also demonstrating strong antioxidants[116–126] and neuroprotective properties[127–132] such as metal chelating and radical scavenging potentials[133–140]. In conclusion, neuronal cell seemed to be the most vulnerable brain cells toward glutamate toxicity. In the absence of astrocyte, neuronal cells are 100-fold more vulnerable to glutamate toxicity. Thus, more studies should be conducted to explore the protective effect of astrocyte on neuronal cell against glutamate toxicity.

Conflict of Interest

The authors declare that there is no conflict of interest in this work.

Author Contributions

The literature review and manuscript writing were performed by H-MY, K-LL and LT-HT.

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