Excitotoxicity induced neuronal deterioration and an up-regulation of EAAT-2 expression via NF-kB pathway with Ceftriaxone: A Review

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Abstract:

Glutamate is the major excitatory amino acid in mammalian nervous system, it has the imperative role in signal transduction process. Oxygen glucose deprived condition like acute ischemic stroke and chronic neurodegenerative disorders such as Alzheimer's, multiple sclerosis and amyotrophic lateral sclerosisare often enhances secretion of glutamate which eventually leads to excitotoxicity.Apart from the deficiency of energy metabolites, an ionic imbalance and ischemic reperfusion will causes oxidative stress followed by release of pro-inflammatory mediators likeTumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) and cytokine such as Inter leukin-1β (IL-1β) and Inter leukin -18 (IL-18). In generalastroglial cells uptake the glutamate through trans-membrane pumps called excitatory amino acid transporter-2 (EAAT-2) thereby it converts glutamate into harmless glutaminein normal physiology of brain.The expression of EAAT-2 protein on plasma membrane of astrocytes will be impaired during acute and chronic neurodegenerative disorders, up-regulation of EAAT-2 in unfavorable conditions like stroke and chronic neurodegenerative disorders could be helpful for neuroprotection. Recent studies revealed that Ceftriaxone can up-regulate EAAT-2 expression via NF-kB pathway. This review will brief the excitotoxicity induced neuronal deterioration, glutamate metabolism and mechanism ceftriaxone induced EAAT-2 expression.

Keywords: Glutamate, Excitotoxicity, ischemic stroke, Ceftriaxone, EAAT-2 expresion.

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Introduction:

Glutamate functions and Clearance

Glutamate is one of the most potential excitatory amino acid inmammalian centralnervous system which plays critical role in generation of action potential, nerve conduction and signal transduction in normal brain physiology **(McEntee and Crook 1993; Weiler et al., 1993;Peng et al., 2011)**.The primary function of glutamate is neuro transmission mediating through ionotropic glutamate receptor such as N-methyl D-aspartate receptors (NMDA),α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

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receptors (AMPA) **(Dingledine et al., 1999)** and also it act as secondary energy resource for neurons in essential times such as hypoglycemic conditions (**Poitry et al., 2000).**

In general, clearance of glutamate is tightly regulated by its release and clearance via excitatory amino acid transporter (EAAT) system. EAAT system has an imperative role in maintenance of low glutamate concentration below to the excitotoxic level where the brain functions will be normal**(Kanai and Hediger, 2003)**.These membrane bounded transporter pumps are usually expressed on various parts of the brain and other body organs like synaptic endings of neuron, astroglial cells, oligo dendrites and retina, there are five (EAAT-1 to EAAT-5) subtypes of excitatory amino acid transporters has been identified till date **(Arriza et al., 1994)**. EAAT-1 mostly expressed on cerebellar region, EAAT-2 abundantly expressed throughout the CNS system, EAAT-3 distributed widely on brain and peripheral tissues, EAAT-4 is primarily found on GABAergicpurkinje cells of cerebellum and EAAT-5 exclusively expresses on retina **(Kanai and Hediger, 2003; Arriza et al., 1994; Beart and O'Shea, 2007)** Among the 5 types of EAAT transporters predominantly EAAT-2 clears the maximum amount of glutamate from the synaptic region after its release, the remaining transporters have not competent to clear the glutamate below to the neurotoxic level where physiological functions of brain will be normal **(Beart and O'Shea, 2007)**.

The mechanisms of glutamate clearance (**Figure 1**) apparently like to be an active transportation, transporter proteins are transports substrate molecule (glutamate) via co and counter transport of ions. The cycle of glutamate clearance begins with formation of a complex with a substrate molecule, $3Na^+$ and $1H^+$ ions at outward facing conformation of EAAT protein and then that complex activates cascade of inward facing conformation eventually substrate, sodium and proton released into cytoplasm of the cell after that transporter returns into outwards facing through counter transport of a K^+ ion and become ready to transport new substrate molecule in the synaptic cleft **(Danbolt et al., 1992; Levy et al., 1998;Robinson, 1998)**. An inadequacy or failure of glutamate reuptake by EAAT system have been proposed to be concerned in a wide range of acute neurotoxic conditions like cerebral ischemia, stroke **(Martin et al., 1997; Torp et al., 1995)** and chronic neurodegenerative disorders such as Alzheimer's disease**(Li et al., 1997)**, Amyotrophic lateral sclerosis **(Rothstein et al., 1992)** and epilepsy **(Tanaka et al., 1997)**.

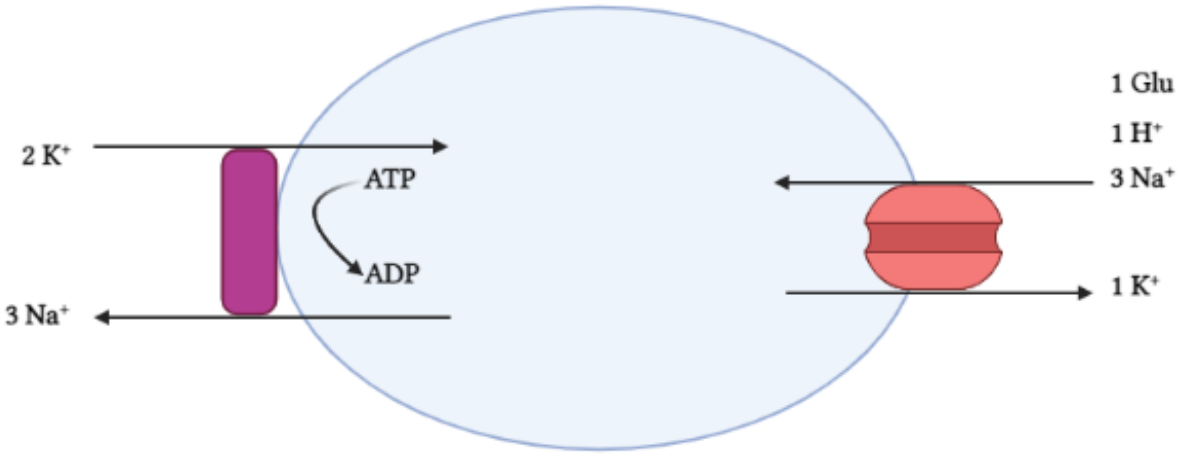

Figure 1. Driving forces involved in clearance of glutamate by astrocytes.

Metabolism of Glutamate

The potential excitatory amino acid glutamate can be metabolized in different pathways of glutamine formation and entry into trycarboxylic acid (TCA) cycle, conversion of glutamate into glutamine occurs in presence of a catalytic enzyme glutamine synthetase, this enzyme loclized to astrocytes and also found in oligodendrocytes but absent in neurons **(Martinez-Hernandezet al., 1977)**.Glutamate transform into 2-oxyglutarate by an oxidative metabolism mediating through either an enzyme glutamate dehydrogenase or transamination process**(Farinelli and nlickles 1992, Sonnewald et al., 1992; Poitry et al., 2000)**. A complete oxidative metabolism of 2-oxyglutarate in TCA cycle produces more than 30 Adenosine triphosphate's (ATP) and greater than 20 ATP's required for uptake of glutamate, these two pathway mechanisms of glutamine conversion as well as 2-oxyglutarate formation vary under different metabolic conditions**(Mckenna et al., 1996; Sonnewald et al.,1997)**. The concentration of extracellular glutamate has the imperative role in conversion of glutamate, for example < 0.2 mM concentration of extracellular glutamate mostly taken into astroglial cells and converts into glutamine by glutamine synthetase and 2-oxyglutarate formed via transaminition reaction, if the concentration of glutamate exceeds more than 0.2 mM in such cases the pathway of glutamine synthetase will be diminished and 2-oxyglutarate produces via de-amination reaction**(Sonnewald et al., 1997)** and also several intermediates like lactate, alanine and glutamine produced by astrocytes during glutamate metabolism and it can serves as energy source**(Poitry et al., 2000)**.

Excitotoxicity induced neurodegenaration

The driving forces for scavenging of glutamate would be supplied from ATP hydrolysis, an ionic imbalance of sodium/Potassium(Na^+/K^+) ions or failure of Na^+/K^+ ATPase pump of Astrocytes due to inappropriate ATP levels will adversely affects the re-uptaking of glutamate **(Zerangue and Kavanaugh,1996; Levy et al., 1998)**. The driving forces will be reduced as a result of increased extra cellular K^+ and intra cellular Na^+ ion concentration, generally transporter proteins (EAAT's) responds to transport the glutamate at constant membrane potential maintained by Na^+ ion gradient in plasma membrane and intra cellular environment of high K^+ and low Na^+ level of astrocyte

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(Szatkowski et al., 1990;Longuemare et al., 1999). The alterations in membrane gradient not only abrupt the reuptake of glutamate but also it reverses the process resultantly glutamate will be leak from the astrocytes viacalcium (Ca^{+2}) dependent mechanism mediating through signaling molecules such as Prostaglandin E2(PGE2) and bradykinins**(Bezzi et al., 1998)**. The reversal release of glutamate occurs in low cellular ATP levels such as cerebral ischemia, the residual ATP often stimulate P_2X_7 receptors and volume sensitive organic anion channels (VSOAC) on astrocytes due to elevated extracellular K^+ions and high intra and extracellular glutamate concentration **(Duan et al., 1999)**.

The oxygen, glucose deprived conditions are generally occurs with ischemic insult in the brain, this is one of the major cause for excitotoxicity induced neuronal dysfunction and death **(Bruijn et al., 2004)**. In addition hypoxic and hypoglycemic circumstances alters the membrane potential of neurons as well as mitochondria which arrests the oxidative phosphorylation and thereby it dysregulate the transportation of glutamate and causes the enhancement of glutamate secretion into intra synaptic region, the accumulated excitatory neurotransmitter rapidly bind ionotropic glutamate receptors like NMDA, AMPA and kinate receptors, activation of these ionotropic glutamate receptors significantly increases extracellular Ca^{+2} levels **(Rothman and Olney, 1995)** which further leads to activate the release of destructive enzymes like protease, lipase and endonuclease's, apart from that reperfusion of blood will bring more amount of oxygen towards injured area and as well as peri-infract region of the brain, the malfunctioning mitochondria could produce oxygen free radicals in presence of excessive oxygen, it occurs as a result of enzyme complexes and leaking electrons from malfunctioning mitochondria, those are interact with molecular oxygen and forms potent superoxide anions eventually it causes oxidative stress**(Gao et al., 2018)**.

The superoxide anions (O_2^-) react with nitrogen oxide (NO) and forms ($OONO^-$)peroxynitrite which promotes the generation of cytotoxic hydroxyl radicals and that are causes to structural alterations in lipids, nucleic acid and proteins of neurons **(Lipton et al., 1993; Yamauchi et al., 1998)**. An energy failure and oxidative stress causes the DNA fragmentation in mitochondria and that disintegrated genetic materials release into cytosol of the cell, fragmented segment of DNA act as damage associated molecular patterns (DAMP's) which further activates the Toll like receptor-9 (TLR-9) **(Zhang et al., 2010)**, eventually it leads to activation of NF-kB signaling pathway thus it promotes production of various pro-inflammatory mediators like tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6)**(Zhang et al.,2014)**. The fragmented mitochondrial DNA may also play vital role in activation of inflammasome NLRP3**(Nakahira et al., 2011)**which also involves in triggering of pro-inflammatory cytokine release like Inter leukin-1β (IL-1β) and Inter leukin -18 (IL-18) that are extremely causes Pyroptotic cell death of neurons **(Fink and Cookson, 2006)**.

Role of Ceftriaxone in Neuroprotection

An excitotoxicity is one of the main cause for neuronal deterioration in acute and chronic neurodegenerative disorders**(Gardoni and Di Luca, 2006)**, tissue plasminogen activators and thrombolitics have been using as a first line agent for the treatment of stroke but the efficacy of these drugs are very limited,usually thrombolytic treatment will be effective upon the administration of drug within 3-4.5 hour after the onset of ischemic stroke symptoms **(Hacke et al., 2008)**. The recent studies have

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revealed that the ceftriaxone is also having the neuoprotective properties, it up-regulates the expression of EAAT-2 on astroglial cells and also has the ability to protect the neurons from excitotoxicity induced neuronal dysfunction in several neurodegenerative diseases.

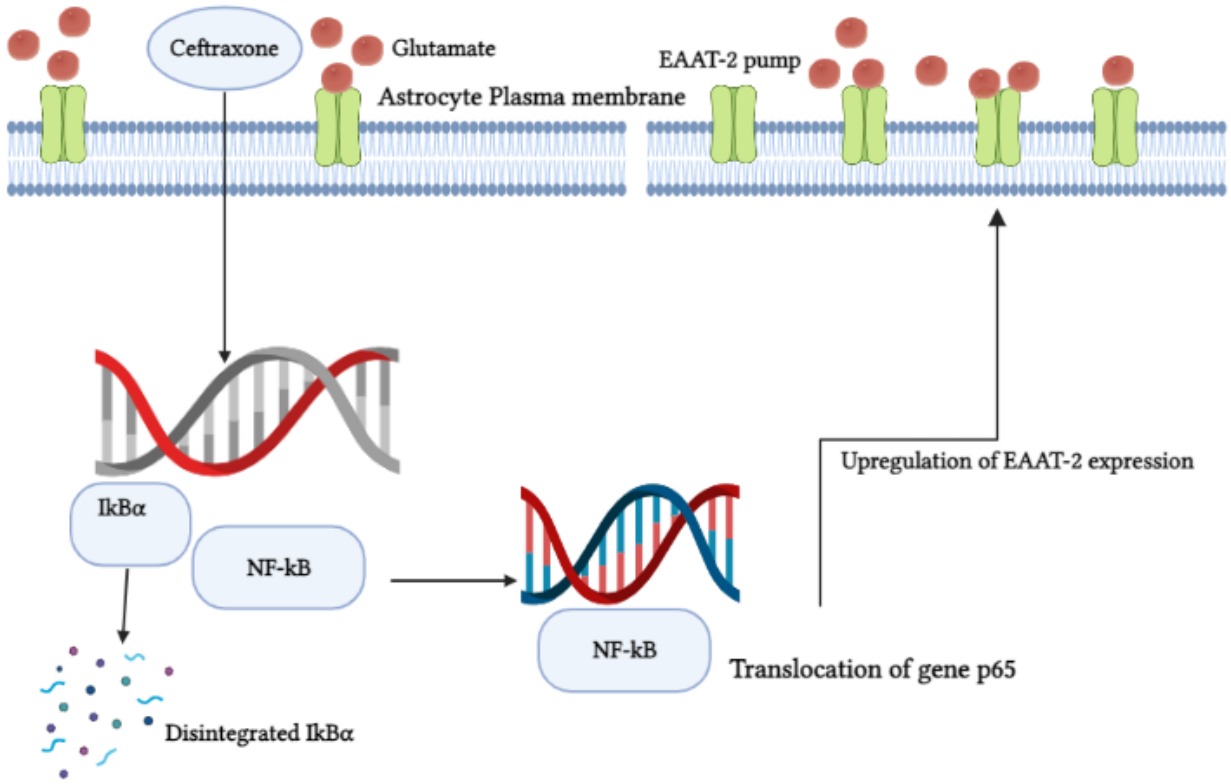

Figure 2. Mechanism of EAAT-2 expression mediating through Ceftriaxone via NF-kB pathway

Third generation cephalosporin, ceftriaxone is **(Figure2)** widely used as a broad-spectrum antibiotic for several bacterial induced pathological conditions and also has been evaluated for the neuroprotectionin suitable*in vitro* neuronal cell line and i*n vivo* animal models**(Rothstein et al.,2005)**. It has been proven that ceftriaxone enhances expression of EAAT-2 in primary human fatal astrocytes (PHFA) via NF-kB signaling pathway, NF-kB plays a crucial role in ceftriaxone mediated EAAT-2 expression and it directly bind to -272 position of EAAT-2 promoter gene which will transcript the transporter proteins**(Lee et al., 2008)**. The activation of NF-kB mediating through degradation of the cytoplasmic inhibitor Inhibitory kappa-B alpha (IkB-α) from NF-kB complex resultantly degraded IkB-α liberates NF-kB to translocate gene p65 of nucleus for the expression of corresponding target genes, these conformational changes up-regulate the expression EAAT-2 over the plasma membrane of astrocytes.

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