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Reducing excitoxicity with glutamate transporter-1 to treat stroke

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Abstract:

The neurotransmitter glutamate is released following ischemic brain damage, and its excitotoxic effects contribute greatly to the development of stroke. Because this release of glutamate occurs within minutes, therapeutic drugs targeting the restriction of glutamate-induced excitotoxicity must be administered quickly following ischemic onset. Here, we evaluate an alternative research approach examining the overexpression of glutamate transporter 1 (GLT1) to reduce infarction and improve behavioral deficits induced by stroke in a rat model of stroke. Recent studies verify the role of glutamate overflow in the pathogenesis of stroke. The experimental approach evaluated glutamate clearance, following ischemia-induced overflow where the GLT had been genetically manipulated to be overexpressed in the ischemic region. A viral vector-mediated gene transfer approach activated the overexpression of GLT1 to successfully reduce ischemia-induced glutamate overflow, decrease cell death, and improve behavioral recovery among animal models. These findings further support the role of glutamate in the pathogenesis of stroke and the upregulation of endogenous GLT1 as a promising approach to protect against the effects of ischemic brain damage caused by glutamate excitotoxicity. This study is a review article. Referred literature in this paper has been listed in the references part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory practice in our research centers and our experiences.

Key words:

Adeno-associated viral-glutamate transporter 1, excitotoxicity, glutamate transporter 1, glutamate, neurodegeneration, neurotransmitter, overexpression, stroke

Introduction

The interruption of blood flow through an arterial vessel in the brain initiates an ischemic stroke, followed by a cascade of cellular and molecular events, eventually resulting in cell death. The initial obstruction results in oxygen depletion, leading to a loss of cellular ATP and alteration of ion homeostasis located at the membrane.[1] This dysregulation of ion homeostasis initiates voltage-dependent calcium channels. The depolarization of the neuronal membrane may result in an enormous discharge of neurotransmitters, including glutamate, into the synaptic cleft and extracellular space for glutamatergic neurons. The extended amount of glutamate in the extracellular space initiates excess stimulation of ionotropic glutamate receptors (iGluRs). This overloaded activation of iGluRs triggers intracellular signaling cascades which yield excitotoxicity and cell death.[2] For years, many have worked to target the restriction of glutamate-induced excitotoxicity as a form of therapy for the treatment of stroke.[3] For example, the acute administration of N-methyl-D-aspartate receptor (NMDAR) or alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) antagonists has proven benefits by reducing degeneration in rodent models of ischemic stroke.[4,5] The blocking of glutamate release or glutamate-mediated postsynaptic excitability has also been discovered to reduce ischemia damage in rat models of stroke.[6,7] These findings suggest that glutamate regulation within the ischemic phase may modify outcomes in animal models of stroke. Despite this, iGluR antagonists in clinical trials have failed to yield successful outcomes and actually possibly impede endogenous neurorepair mechanisms.[8]

Therapeutic drugs targeting the restriction of glutamate-induced excitotoxicity must be administered quickly following ischemic onset because the release of glutamate occurs within minutes. Alternatively, the glutamate clearance from the extracellular space could be enhanced to avoid excess iGluR activation.

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Of note, five subtypes of the sodium-dependent excitatory amino acid transporters (EAAT1-5) exist that separate themselves based on their local and cellular expression and capability to transport glutamate.[9] Transient middle cerebral artery occlusion (MCAo) reduces glutamate transporter 1 (GLT1) (EAAT20, a predominantly glia-expressed EAAT).[10,11] In addition, reducing GLT1 messenger RNA by antisense knockdown intensified neuronal cell death triggered by transient MCAo.[12] Particular molecules such as beta-lactams have been demonstrated to augment the expression of the GLT in the brain.[13] For example, Chu *et al*. confirmed that intraperitoneal administration of ceftriaxone for 5 successive days preceding stroke reduced infarct volume and improved behavior outcomes.[14,15] Wang *et al*. further explores this approach as a treatment for stroke by augment glutamate clearance by producing an adeno-associated viral vector to express the rat GLT1 complementary DNA (cDNA) (AAV-GLT1). The intracortical delivery of AAV-GLT1 proved to reduce ischemic damage and the degree and length of ischemia-induced glutamate overstimulation induced by MCAo.

Adeno-associated Viral-Glutamate Transporter 1 Decreases Infarction and Promotes Behavioral Recovery

In a study by Wang *et al*., they demonstrated that rats receiving AAV-GLT1 showed significant improvement in behavioral recovery and infarct volume. Initially, increased glutamate clearance rate in nonstroke rat brain confirmed the expression of AAV-GLT1, as measured by *in vivo* amperometry. Three weeks before 60 min MCAo, AAV-GLT1 was injected into the future cortical region of infarction. Tissue damage was then evaluated at 1 and 2 days following MCAo using TUNEL and TTC staining, respectively. Then, the investigators performed behavioral testing at 2, 8, and 14 days after stroke. Microdialysis showed that rats receiving AAV-GLT1 demonstrated a drastic decrease in the length and magnitude of extracellular glutamate during the 60 min MCAo. Pretreatment with AAV-GLT1 significantly reduced the brain infarction and DNA fragmentation compared to AAV-green fluorescent protein (GFP) injections. Furthermore, those animals that received AAV-GLT1 displayed substantial improvement in behavioral recovery poststroke compared to the AAV-GFP group as well.

Adeno-associated Viral-Glutamate Transporter 1 Reduces Ischemia-induced Glutamate Overflow in Lesioned Cortical Region

Wang *et al*. also showed that the focal overexpression of the GLT, GLT1, drastically reduced glutamate efflux caused by ischemia. An *in vivo* microdialysis measured this during and after the 60 min MCAo. They detected an increase in extracellular glutamate until 20 min, following MCAo initiation for both AAV-GFP and AAV-GLT1 treated animals. This level of glutamate overflow was shown to be reduced over time in animals that received the pretreatment of AAV-GLT1. In addition, animals receiving AAV-GLT1 exhibited a significant reduction (48%) in TUNEL staining compared to the AAV-GFP group. These data further suggest that augmenting GLT1 expression is protective against ischemic damage.

Consideration for Future Clinical Development

The excess amount of glutamate in the extracellular space following ischemia has been acknowledged for over 25 years.[16] This overflow initiates an over-activation of ionotropic postsynaptic GluRs, such as NMDA and AMPA. The amplified influx of calcium triggers intracellular signaling cascades due to the activation of iGluRs.[2] Several studies have demonstrated that NMDAR or AMPAR antagonistic administration lessens ischemic damage in rodent stroke models.[4,5] In addition, Shen *et al*. have shown that neural degeneration can be reduced in stroke rats by specific pharmacological agents that prevent glutamate-mediated postsynaptic excitability.[6,7] The current study verifies the role of glutamate overflow in the pathogenesis of stroke.[17] Furthermore, this study explores gene therapy as a promising approach to reduced ischemia-induced excitotoxicity. An AAV vector was used to deliver the rat GLT1 cDNA to the rat cortex, and a significant reduction of infarction and ischemia-induced glutamate overflow was observed. These data indicate that increasing GLT1 expression confers a protective effect against MCAo-induced ischemia. A progressive improvement should also be noted here as the group recorded the recovery of neurological deficits (Bederson's score) and body asymmetry in animal models that received pretreatment of AAV-GLT1 compared to control AAV-GFP. Taken together, the behavioral observations also support that the overexpression of AAV-GLT1 at the time of ischemia is able to reduce the glutamate overflow and prevent against excitotoxicity.

Previous studies have documented that pretreatment with GluR antagonists reduces neurodegeneration in the ischemic animal brain.[18] It has also been found that excitatory amino acids have trophic response to developing neurons.[19,20] Moreover, administration of the NMDAR blocker MK-801 inhibited the increased neurogenesis in the hippocampus of rats following MCAo.[21] These findings indicate two conflicting responses of GluR antagonists in stroke. Interestingly, administration of GluR antagonists reduces neurodegeneration in the ischemic area, yet decreases neuroregeneration in nondamaged areas. These opposite reactions may produce failing results in clinical trials using GluR antagonists in stroke patients.[22] A possible approach would be decreasing glutamate concentrations or reactions locally in the affected region while also preserving the endogenous glutamate function in regions for neurogenesis, including the subventricular zone.[23] Harvey *et al*. demonstrated this approach where the overexpression of GLT1 in rat cortex reduced infarction and improved neurological deficits.[17]

In addition, their experimental data indicated that increasing the capacity to clear extracellular glutamate through gene transfer provides beneficial outcomes against ischemia-induced glutamate release. This approach had been accomplished by the GLT being genetically manipulated to be overexpressed in the ischemic region. While previous clinical trials have failed to produce successful results regarding systemic glutamate clearance, the current trial suggests that focal glutamate clearance can be beneficial. For example, certain with nonavoidable risk factors such as heart attacks or previous surgery people at risk

for could likely benefit from increased GLT-1 levels cause by focal pharmacological[13-15] or genetic manipulation as the less invasive selective delivery of genes develops.

Conclusion

The present study demonstrates the success of a viral vector-mediated gene transfer approach to initiate the overexpression of GLT1 to decrease ischemia-induced glutamate overflow, reduce cell death, and improve behavioral recovery. These findings further support the upregulation of endogenous GLT1 as a promising approach to protect against the detrimental effects of stroke.[13-15] Future studies should further develop the use of GLT1 in making this selective gene therapy, a more viable approach reduces the brain damage resulting from glutamate excitotoxicity.

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Conflicts of interest

There are no conflicts of interest.

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