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Mini review

# Astrocytic therapies for neuronal repair in stroke

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## HIGHLIGHTS

- Astrocytes are an under-investigated area of therapeutics for stroke recovery.
- Changes in astrocytes may help or hurt neurons acutely after stroke.
- Astrocytic responses promote or inhibit neural repair long-term.
- Targets and times of astrocyte-directed intervention in stroke are discussed.

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## ABSTRACT

Stroke is a leading cause of disability and death worldwide. Much of the work on improving stroke recovery has focused on preventing neuronal loss; however, these approaches have repeatedly failed in clinical trials. Conversely, relatively little is known about the mechanisms of repair and recovery after stroke. Stroke causes an initial process of local scar formation that confines the damage, and a later and limited process of tissue repair that involves the formation of new connections and new blood vessels. Astrocytes are central to both scar formation and to tissue repair after stroke. Astrocytes regulate the synapses and blood vessels within their cellular projections, or domain, and both respond to and release neuroimmune molecules in response to damage. Despite this central role in brain function, astrocytes have been largely neglected in the pursuit of effective stroke therapeutics. Here, we will review the changes astrocytes undergo in response to stroke, both beneficial and detrimental, and discuss possible points of intervention to promote recovery.



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## 1. Introduction

Stroke is a leading cause of disability and death worldwide, affecting almost 800,000 people every year in the United States alone [14]. Eighty-seven percent of strokes are ischemic, in which blood flow to the brain is reduced; the remaining 13% are hemorrhagic, in which a vessel ruptures and blood accumulates in the brain. Because of its higher prevalence and the widespread

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availability of validated animal models, most research efforts have focused on ischemic stroke; this review will take the same approach. Many neurons die within a few hours after stroke; therefore, considerable effort has been devoted to the development of drugs that would confer neuroprotection when delivered shortly after stroke. While this strategy has shown promise in animal models, it has failed in clinical trials [38]. One possible explanation for this failure is that neuronal survival alone may be insufficient to promote recovery. Therefore, in recent years there has been an increased focus on the roles of astrocytes in stroke.

Astrocytes play a number of key roles in a properly functioning nervous system. Astrocytes are crucial in coordinating changes in vascular tone in response to neuronal activity; removing excess glutamate from the synaptic cleft, limiting transmitter spillover and preventing excitotoxicity; aiding in the formation and integrity of the blood–brain barrier (BBB); promoting synaptogenesis; and responding to and releasing pro- and anti-inflammatory molecules [42]. In stroke, all of these activities are affected. Here, we will review the changes astrocytes undergo after stroke, the beneficial and detrimental consequences these changes have for recovery, and the ways in which astrocytic responses may be modulated to promote repair and recovery. Because treatments administered before or at the time of ischemia are impractical for clinical translation, we will focus on systems in which post-ischemic modulation shows promise.

The studies described here encompass several stroke models [11]. In cell culture, stroke is modeled through oxygen/glucose deprivation (OGD). The primary model used in vivo is middle cerebral artery occlusion (MCAO) in rodents, which produces focal ischemia. MCAO can be transient (tMCAO, generally ranging from 30 min to several hours), or permanent (pMCAO). Another common model is photothrombotic stroke: a photosensitive dye is injected intraperitoneally, followed by localized stereotaxic illumination through the skull. This causes photooxidation within blood vessels, resulting in localized damage. Other models include microsphere injection, which causes a number of small infarcts throughout the brain, and four-vessel occlusion, in which the vertebral arteries are electrocauterized and the carotid arteries are temporarily clamped, producing global ischemia [34]. Astrocytic responses play crucial roles in all of these models, as discussed below. While we will not discuss hemorrhagic stroke here, due to its less prevalent and less studied nature, several recent papers have begun to explore the role of astrocytes in hemorrhagic stroke models [29,43].

## 2. Reactive astrocytosis

Reactive astrocytosis refers to the changes astrocytes undergo in response to injury or disease. It is a complex and gradated process, ranging from minor changes in gene expression to cell hypertrophy to astrocyte proliferation and scar formation [41]. The process begins almost immediately after injury. While minor forms of reactive astrocytosis can resolve over time, more severe changes, such as scar formation, can be permanent. The extent to which reactive astrocytosis is beneficial vs. detrimental is still an active area of investigation: some functions limit damage and promote recovery, while others exacerbate injury [41]. Different injury models produce divergent changes in astrocytes: a recent genomic analysis of reactive astrocytes isolated after tMCAO or lipopolysaccharide (LPS) injection, a model of neuroinflammation, found that over 50% of the genes induced are not shared between models [49]. Furthermore, the patterns of reactivity revealed interesting differences: in general classification terms, the gene expression profile of MCAO-reactive astrocytes are largely protective, expressing neurotrophic factors and cytokines, while LPS-reactive astrocytes are largely destructive, upregulating genes that destroy synapses. Similarly, exposure of cultured astrocytes to cytokines triggers an increase in secretion of synapse-destroying complement proteins, chemokines, and extracellular matrix proteins, as measured by mass spectrometry [23].

There is also evidence that reactive astrocytosis is detrimental, or at least not necessary, for stroke recovery. For example, knockout of the neuronal MHC class I receptor PirB improves behavioral recovery from tMCAO by decreasing cell death and increasing neuronal plasticity and axonal outgrowth [3]; this correlates with a decrease in reactive astrocytosis, indicating that a reactive phenotype may not be necessary for neuronal plasticity after stroke. Similarly, Hsp72 overexpression, which is protective in stroke, decreases astrocyte reactivity and complexity [7]. However, astrocytes are involved in a variety of processes that change after stroke; it may be possible to augment astrocytic events that promote recovery, while inhibiting responses that prevent recovery.

## 3. Excitotoxicity

One early potential points of intervention in stroke is reduction of excitotoxicity in the peri-infarct region. The peri-infarct region is the tissue immediately adjacent to the infarct core that can be incorporated into the core over time but also has potential to recover. The extent to which the peri-infarct region withstands further damage likely influences stroke severity and recovery potential [52]. One way in which ischemic damage spreads throughout neuronal tissue is via excitotoxicity, a process by which excess extracellular glutamate leads to neuronal $Ca^{2+}$ influx, ultimately causing neuronal death [24]. Astrocytic glutamate transporters can buffer and sequester glutamate, reducing excitotoxicity. The astrocytic transporter glutamate transporter 1 (GLT-1) is downregulated in response to stroke [35]. Further decreasing GLT-1 levels via siRNA increases infarct size in tMCAO in rats [36], while viral upregulation of GLT-1 decreases infarct size and promotes behavioral recovery [16]. These studies suggest that astrocytes may provide partial protection from excitotoxicity and peri-infarct damage via GLT-1-mediated glutamate buffering, and that this effect has yet to reach a ceiling. Therefore, a different approach to targeting stroke-induced excitotoxicity may be to increase GLT-1 expression.

One possible way to increase GLT-1 levels is with tamoxifen, an estrogen receptor modulator that is used to treat breast cancer. Tamoxifen administration three hours after the onset of tMCAO in rats causes a 90% decrease in infarct size and reduced behavioral deficits [51]. Another possible GLT-1-inducing drug is the amyotrophic lateral sclerosis drug riluzole, which is associated with increased GLT-1 expression [10]. Riluzole reduces infarct size when administered two hours after tMCAO in mice, although it does not alter pMCAO [44]. In fact, riluzole is currently being used in clinical trials in another type of CNS injury, spinal cord injury. Although full results from the phase I trial are not yet available, preliminary results suggest that riluzole may be a promising option, strengthening the possibility that riluzole may also prove useful in stroke [45].

## 4. Astrocyte proliferation

One sign of severe reactive astrocytosis is astrocyte proliferation [41]. With time, these astrocytes can help form a scar around the infarct, sealing off the site and preventing the spread of damaging molecules to intact tissue; however, this scar can also limit the extent of axonal outgrowth and regeneration. The beneficial or detrimental effects of astrocyte proliferation remain a subject of active study. In some cases astrocyte proliferation has been shown to be protective. Newly-born subventricular zone astrocytes express high levels of thrombospondin (Thbs) 4, a secreted

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