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Astrocytic therapies for neuronal repair in stroke

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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.

Keywords

Stroke; astrocytes; neural repair

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 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 minutes 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 extracellular matrix glycoprotein. These astrocytes migrate to the peri-infarct cortex when the infarct is produced in developmentally immature brains and are necessary for microvessel integrity, in a Thbs-4-dependent fashion [9]. These results suggest that astrocyte proliferation has protective effects beyond scar formation.

In other studies, however, inhibition of proliferation has been associated with recovery. After stroke an extracellular matrix protein, perlecan, is cleaved into a functional fragment, domain V. Treatment with domain V twenty-four hours after tMCAO in rats reduces infarct size and promotes angiogenesis and behavioral recovery [27]. This effect may be mediated by astrocytes: astrocytes bind domain V, which triggers decreased astrocyte proliferation, changes in astrocyte morphology, increased astrocyte migration, and increased astrocytic release of nerve growth factor [4]. These results suggest that minimizing astrocyte proliferation, while increasing the ability of astrocytes to enter a hypertrophic state and migrate towards a VEGF gradient, may be beneficial; proliferation may not be necessary if sufficient post-mitotic astrocytes can reach the infarct site.

5. Neuroinflammation

Astrocytes both respond to and produce immune molecules like cytokines and chemokines, of both pro- and anti-inflammatory natures [6]. Of the genes increased by fourfold or more in reactive astrocytes one day after tMCAO, a quarter of them are involved in immune response [49]. Interestingly, astrocytic dopamine receptors play a key role in the control of neuroinflammation [40]. Activation of the astrocytic dopamine D2 receptor (DRD2) suppresses neuroinflammation in injury by inducing production of the immunosuppressant molecule αB-crystallin, while astrocyte-specific deletion of DRD2 exacerbates cytokine production and damage. Increasing dopamine through levodopa administration has previously been associated with improved motor function in stroke patients [39], although other studies in humans have shown mixed results [2]. In rats, levodopa administration starting two days after tMCAO improves functional recovery without decreasing infarct size, possibly by triggering upregulation of glial-derived neurotrophic factor in peri-infarct astrocytes [26]; in this case, levodopa's effects were attributed to activation of astrocytic dopamine receptor D1, and the effect of levodopa administration on the inflammatory milieu after stroke was not examined. These results suggest that targeting astrocytic dopamine receptors may be a viable treatment option relatively late after stroke, potentially decreasing neuroinflammation as well as increasing production of trophic factors.

6. Angiogenesis and BBB repair

Astrocytes may also mediate stroke recovery through increased angiogenesis and BBB repair. The BBB is disrupted for up to two weeks after stroke [1]; while stroke induces formation of new vessels, many are leaky and do not persist long-term [48]. Astrocytic secretion of Sonic hedgehog (Shh) promotes BBB formation and integrity *in vivo*, in both development and adulthood [5], while Shh release from cultured astrocytes mediates angiogenesis after OGD [19].

Shh induces upregulation of astrocytic angiopoietin-1, which is secreted and causes upregulation of tight junction proteins in endothelial cells, repairing BBB permeability [46]. Angiopoietin-1 is thought to be necessary to vessel maturation [32]; therefore, it is an attractive target in stroke, when vessels form but do not persist. Most studies upregulating angiopoietin-1 have relied on injection of angiopoietin-1-expressing cells or viral vectors [32]. An agonist to the angiopoietin-1 receptor, Tie-2, has recently been developed [25]; it would be interesting to explore its effects in post-stroke vessel maturation. This might offer a relatively late treatment option, as extensive leaky vessels have been found 30 days after tMCAO in rats [48].

In order to fix new, leaky vessels, new vessels must first be formed. High-mobility group box 1 (HMGB1), a DNA binding protein that acts as an inflammatory mediator, is upregulated and released from astrocytes after tMCAO. HMGB1 promotes expansion of endothelial progenitors, which enhance angiogenesis; this effect can be blocked by injection of HMGB1 siRNA five days post-stroke [17]. These results suggest that that the HMGB1-mediated effect on angiogenesis may present a viable target for enhancing recovery several days after stroke onset. Indeed, any HMGB1-directed therapies may be best suited to a later timepoint, as acute HMGB1 release in ischemia promotes necrosis and inflammatory damage [18]. Clinically, HMGB1 levels in stroke patients are being evaluated at multiple timepoints post-stroke (NCT01705353); these results may be helpful in designing HMGB1-directed therapies.

7. Synaptogenesis and axonal sprouting

Astrocytes are also important in the formation of the new synapses that are necessary for stroke recovery. In addition to the role of thrombospodin-4 in astrocyte proliferation and vessel stability after stroke [9], other Thbs proteins are involved in neural repair processes. Astrocytic secretion of Thbs 1 and 2 promotes synaptogenesis [12], and is upregulated after pMCAO in mice. Additionally, Thbs-1/2 knockouts show defects in post-stroke synaptogenesis and axonal sprouting, indicating that astrocytic upregulation of these proteins is crucial for neural repair [28]. Although thrombospondins are anti-angiogenic in other systems, no effect was found in stroke. Indeed, Thbs-4 knockout promotes microvessel hemorrhage after stroke [9], although whether this is due to loss of astrocyte proliferation or a Thbs-4 effect on BBB stability is unclear. Therefore, increase of Thbs secretion from astrocytes could prove effective in stroke recovery. Because of thrombospondins' anti-angiogenic properties, mechanisms to increase Thbs expression or activity have been investigated in cancer biology. Successful strategies include repeated, low-dose chemotherapy treatment and delivery of mimetic peptides [50]. These approaches may be worth investigating in stroke; while stroke recovery benefits from an increase, not decrease, in angiogenesis, the lack of effect of Thbs-1/2 knockout on post-stroke angiogenesis suggests that a Thbs-based approach may have differential effects in the nervous system versus tumors.

Several neurotrophic growth factors are secreted from astrocytes. Brain-derived neurotrophic factor (BDNF) gene expression is upregulated in reactive astrocytes one day after tMCAO [49], and BDNF protein levels are increased eight days after infarction by microsphere injection [8]. Astrocytic release of glial-derived neurotrophic factor (GDNF) has also been shown to be protective in culture, and may be related to astrocytic activation of the adrenergic receptor α2A [47] or, as mentioned earlier, the dopaminergic receptor D1 [26], suggesting that pharmacologic activation of either of these systems may promote stroke recovery.

tMCAO triggers a rapid and long-lasting (>2 weeks) upregulation of another astrocytic growth factor, ciliary neurotrophic factor (CNTF) [22]. This growth factor is expressed only in astrocytes in the CNS, at low basal levels. Interestingly, the stroke-induced upregulation of CNTF may be due to the loss of neuron-astrocyte contact: under physiologic conditions, CNTF is repressed through direct neuron-astrocyte contact. When these contacts are lost after neuronal death in stroke, CNTF is rapidly increased. Stroke-induced neurogenesis is absent in CNTF knockout mice [21], which may indicate a negative-feedback role for CNTF during repair: loss of neuron-

astrocyte contact triggers CNTF upregulation, in turn promoting neurogenesis. Once new neurons, or processes of surviving neurons extending into peri-infarct tissue, make contact with astrocytes, CNTF would again be repressed. This regulation makes CNTF an intriguing possible target for astrocytic promotion of axonal outgrowth in stroke. A CNTF implant is currently being evaluated in a phase I trial for treatment of ischemic optic neuropathy (NCT01411657); should the implant prove efficacious, CNTF will be an even more attractive target for stroke recovery.

Axonal outgrowth is a mechanical process that requires motility and spatial restriction of signaling proteins, which can involve lipid rafts. Lipid rafts are cholesterol-enriched sections of membrane [15]. In the CNS, cholesterol is largely produced by astrocytes. Cholesterol-dependent mobilization of lipid rafts may help axonal outgrowth after stroke. Stroke-induced increased expression of the cholesterol-binding sigma-1 receptor in astrocytes is beneficial; treatment with a sigma-1 receptor agonist two days after MCAO enhances behavioral recovery and neurite outgrowth without decreasing infarct size, suggesting a neural repair, rather than neuroprotection, mechanism [37]. This may be due to increased export of cholesterol to neurons via the sigma-1 receptor. Similarly, increasing high-density lipoprotein cholesterol (HDL-C) by administration of the liver X receptor agonist GW3965 twenty-four hours after tMCAO in mice improves recovery, causing synaptogenesis and angiogenesis [13]. Low HDL-C levels have long been known to increase stroke risk, but also correlate with decreased stroke recovery in patients [30]; this may partially be explained by the importance of cholesterol in synaptogenesis, and suggests that there may be benefit to manipulating HDL-C levels after stroke.

However, astrocytes can also inhibit neural repair after stroke. For example, ephrin-A5 is upregulated in peri-infarct reactive astrocytes seven days after pMCAO, and acts to block neuronal outgrowth and minimize repair [33]. Inhibition of ephrin-A5 seven days after stroke promotes axonal outgrowth and behavioral recovery. These results suggest that ephrin-A5 manipulation may be a therapeutic target with a long treatment window. Several proteins and small molecules have been developed to modulate Eph receptor signaling [31]. By determining which EphA receptors are required for the inhibitory effect of astrocytic ephrin-A5 on axonal outgrowth, it may be possible to take advantage of existing Eph receptor modulators to promote recovery.

8. Astrocyte transplantation

To explore the role of astrocyte-specific transplants in ischemia, Jiang et al [20] differentiated human ESCs into astrocytes using two different progenitor populations, Olig2+ versus Olig2- neural progenitor cells, and transplanted them into rats that had undergone four-vessel occlusion to produce global cerebral ischemia six hours previously. Transplantation with either group decreases neuronal loss and improves behavioral recovery; however, the Olig2+-astrocytes are more efficacious than the Olig2- population, possibly due to higher BDNF production by the Olig2+ population. Interestingly, Olig2+ astrocyte transplantation also results in increased synaptogenesis, as measured by synapsin-1 staining. Together, these results suggest that transplantation of specific astrocytic populations may effectively promote neural repair after ischemia.

9. Conclusions

Astrocytes undergo a number of changes in response to stroke, affecting virtually all astrocytic functions. Many of these changes are beneficial and improve recovery, including the release of angiogenic molecules and upregulation of growth factors and other synaptogenic proteins. However, other astrocytic changes are clearly detrimental to recovery, including increased excitotoxicity via the downregulation of GLT-1 and the release of ephrin-A5 to inhibit axonal outgrowth (Figure 1). Still other astrocytic changes have unknown or ambiguous roles in recovery, such as the production of cytokines and astrocyte proliferation. Any potential future

astrocyte-directed therapies in stroke treatment may depend on maximizing the existing beneficial effects while inhibiting astrocytes' detrimental functions.

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Figure 1: Timeframe for astrocytic therapies. There are a number of promising potential therapeutics that would target astrocytic functions after stroke, with different anticipated outcomes. Not all of these therapeutics would be appropriate at all timepoints; here, we divide them into approximate therapeutic windows.

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