MINIREVIEW

Brain angiogenesis in developmental and pathological processes: neurovascular injury and angiogenic recovery after stroke

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Introduction

The neuron has traditionally been viewed as the most important cell type within the mammalian central nervous system (CNS) because it is the fundamental unit for neurotransmission. Death or dysfunction in neurons leads to loss of brain function in many diseases. Therefore, saving neurons, i.e. neuroprotection, should be a logical therapeutic goal, especially in stroke. Over the past many decades, impressive advances have been made in dissecting stroke mechanisms involving excitotoxicity and ionic imbalance, oxidative and nitrosative stress, neuroinflammation and apoptotic-like pathways in neurons. However, clinically effective neuroprotectants have not yet been discovered. Although there are many reasons why stroke neuroprotection trials have not succeeded [1–3], it is possible that a singular focus on saving neurons alone might not be sufficient.

In recent years, the concept of the ‘neurovascular unit’ has emerged as a new paradigm for understanding pathophysiologic responses in brain after stroke are highly complex. Thus far, a singular focus on saving neurons alone has not revealed any clinically effective neuroprotectants. To address this limitation, the concept of a neurovascular unit was developed. Within this conceptual framework, brain function and dysfunction are manifested at the level of cell–cell signaling between neuronal, glial and vascular elements. For stroke, coordinated responses at the neurovascular interface will mediate acute as well as chronic events in ischemic and hemorrhagic brain tissue. In this minireview, we briefly survey two representative examples of neurovascular responses in stroke. During the early acute phase of neurovascular injury, blood–brain barrier perturbations should predominate with key roles for various matrix proteases. During the delayed phase, brain angiogenesis may provide the critical neurovascular substrates for neuronal remodeling. In this minireview, we propose the hypothesis that the biphasic nature of neurovascular responses represents an endogenous attempt by damaged parenchyma to trigger brain angiogenesis and repair. This phenomenon may allow acute deleterious signals to transition into beneficial effects during stroke recovery. Understanding how neurovascular signals and substrates make the transition from initial injury to angiogenic recovery will be important if we are to find new therapeutic approaches for stroke.

Abbreviations

BBB, blood–brain barrier; CNS, central nervous system; EPC, endothelial progenitor cell; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; NMDA, N-methyl-D-aspartate; t-PA, tissue-plasminogen activator; VEGF, vascular endothelial growth factor.
the pathology of CNS disease, including stroke [3–7] (Fig. 1). This modular concept is defined at an intercellular level that comprises dynamic interactions between cerebral endothelial cells, glia, neurons and the extracellular matrix. Dissecting these various signals and substrates within the neurovascular unit may reveal opportunities for developing novel therapeutic targets for CNS disease. Perhaps, preventing neuronal death per se may not be enough. In order to truly rescue brain tissue and function, one may have to rescue all the complex signals and interactions between a network of multiple cell types, including neurons, astrocytes and microvascular endothelial cells.

Stroke (also called a brain attack) refers to a heterogeneous spectrum of conditions caused by the occlusion or hemorrhage of blood vessels supplying the brain, and is one of the major causes of death and disability in developed countries [3]. The initial vascular event leads to energy loss, which triggers activation of multiple brain cell death pathways. In addition to brain injury responses, regenerative responses are also activated by stroke, such as vascular remodeling, angiogenesis and neurogenesis. The full spectrum of pathophysiology after stroke is complex and readers are referred to many excellent reviews in the field [4–6,8–10]. In the context of all these multicellular perturbations however, it may be useful to ask whether neurovascular responses after stroke can be reinterpreted in the context of angiogenesis in the brain. Is it possible that some of the acute neurovascular events in the brain after stroke represent an endogenous attempt by the brain to prepare the substrates necessary for angiogenesis and recovery? In this minireview, we survey a few key events in the neurovascular unit, including blood–brain barrier (BBB) perturbations, matrix proteases, coupling between neurogenesis and angiogenesis, and endothelial progenitor cells (EPCs). We examine the idea that neurovascular responses underlie a transition from acute injury to delayed repair as the brain begins to initiate endogenous angiogenesis that facilitates neuronal plasticity and remodeling. A systematic understanding of these responses may eventually lead us to discover new targets for treating brain injury after stroke.

**Early neurovascular damage in stroke**

In the core of the ischemic territory, the initial vascular event rapidly leads to severe energy loss, and so neuronal death may occur too rapidly for treatment. However, surrounding the core is an area of mild-to-moderate vascular compromise called the penumbra. Within this penumbral area, energy deficits are not as severe and it is thought that neuronal death occurs via active cell death mechanisms [11–14]. By understanding these neuronal death pathways, it is hoped that one can design methods to block cell death after stroke. Nevertheless, focusing only on intraneuronal mechanisms may lead us to miss many other critical interactions of neurovascular damage (Fig. 2).

One of the most important facets of early neurovascular damage is manifested as perturbations in BBB function. Interactions between brain endothelial cells, astrocytes and adjacent neurons all support BBB function. After cerebral ischemia, intercellular signaling within the neurovascular unit becomes disrupted so that the BBB function is dysfunctional. BBB disruption leads to vasogenic cerebral edema and hemorrhage that eventually exacerbates long-term disability. To date, numerous deleterious mediators have been reported to be relevant to early neurovascular damage (see Green [8] and Lo et al. [3] for more detailed reviews). Hypoxia may alter the regulation of critical tight junction proteins [15,16], changes in calcium control may disrupt the signaling between astrocytes and endothelial partners [17,18], and activation of inflammatory pathways in damaged endothelium might also open the BBB [19,20].

In recent years, dysregulation of neurovascular proteases has been implicated as central in neurovascular injury after stroke. In particular, the matrix metalloproteinase (MMP) family of extracellular proteases has been very well studied. MMPs comprise a family of zinc endopeptidases with major roles in the physiology and pathology of the mammalian CNS. To date, MMP-2 (gelatinase A), MMP-3 (stromelysin 1), MMP-7 (matrilysin), MMP-9 (gelatinase B) and MMP-13 (collagenase-3) are known to contribute to infarct extent and/or BBB disruption after stroke [21–25]. There are three main reasons why MMPs are important in stroke. First, MMPs can degrade the extracellular matrix that comprises the basal lamina, thus damaging the BBB directly. Second, proteolysis of the
neurovascular matrix can also trigger anoikis-like mechanisms of neuronal death [26]. Third, MMPs are upregulated by tissue-plasminogen activator (t-PA), which is the only US Food and Drug Administration-approved thrombolytic treatment for acute ischemic strokes. The reader is encouraged to seek more detailed reviews describing the interactions between MMPs and stroke-induced brain damage [27,28]. Here, we focus on the relationship between t-PA and MMPs, insofar as these clinical correlates may be especially important for stroke therapy in humans.

Thrombolysis with t-PA is logical for acute ischemic stroke in terms of dissolving clots and reperfusing brain tissue. However, reperfusion therapy can sometimes be negated by serious complications involving cerebral edema and hemorrhage. Accumulating evidence suggests that MMP-9 activation is closely related to those side effects of t-PA. In a hypertensive rat model of thromboembolic focal cerebral ischemia, early treatment with t-PA was beneficial, but delayed t-PA administration worsened outcomes because it appeared to accelerate MMP-9 activation [29]. Activation of MMP-9 by t-PA seemed to correlate with hemorrhagic conversion and edema. Using a combination therapy with the broad-spectrum MMP inhibitor BB-94 plus t-PA showed significantly reduced hemorrhage volumes compared with those that received t-PA alone, suggesting that MMPs are involved in the mechanism of t-PA-associated hemorrhage. This pharmacologic evidence was subsequently supported by a genetic study, wherein t-PA knockout mice were used to demonstrate that both endogenous and exogenous t-PA were related to MMP-9 activation in ischemic brain [30]. Furthermore, the cellular mechanisms of t-PA-induced MMP-9 upregulation are now beginning to be dissected. In endothelial cell cultures, t-PA upregulated MMP-9 via signaling through the low-density lipoprotein receptor-related protein [31]. In vivo, t-PA was shown to also directly open the BBB in models of focal ischemia with complex signaling actions involving the platelet-derived growth factor and low-density lipoprotein receptors [32,33]. These experimental findings are now beginning to be supported by clinical data. In acute stroke patients, t-PA appeared to be correlated with elevations in plasma levels of MMPs [34], and these higher MMP levels seem to be somewhat predictive of worsened neurological outcomes [35,36].

Despite the promising data, much more work needs to be done. Many experimental and clinical studies have focused on MMP-2 and MMP-9 because they interact with t-PA and there are simple and reproducible assays to detect their levels via gelatin zymography or ELISAs. Of course, other MMPs may also be involved because these proteases are known to function as a network. Using knockout mice, a recent study demonstrated that MMP-3 is important mediator for t-PA-induced intracranial bleeding in mouse models of focal stroke [37]. In this model, co-treatment with the broad-spectrum MMP inhibitor GM6001 effectively reduced intracranial bleeding. More recently, it was reported that minocycline might be a potential agent to downregulate t-PA-induced MMP-9 activation and ameliorate t-PA-associated hemorrhage during reperfusion therapy in stroke [38]. The translational attractiveness of this approach lies in the fact that minocycline can be easily used in humans.

Taken together, the accumulating experimental and clinical data suggest that MMPs (and perhaps other extracellular proteases) may mediate neurovascular injury during the acute stages of stroke. In this regard,
targeting these neurovascular proteases may serve as a powerful combination therapy with t-PA thrombolysis [39]. However, an important caveat here is that conserved responses in this regard may also play differential roles during later stages of stroke evolution. Is it possible that acute mechanisms of neurovascular injury are altered to beneficial neurovascular remodeling over the course of stroke recovery? Here, we propose the hypothesis that these acute neurovascular events may, in fact, represent an endogenous attempt by the brain to initiate angiogenic recovery. Altered calcium signaling between astrocytes and the corresponding endothelial cells may underlie proximal triggers for vascular remodeling. Loosening of tight junctions occurs as endothelial cells disengage in preparation to move; and of course, upregulation of extracellular proteases such as MMPs is required for angiogenesis and vasculogenesis. Attempts to retard any of these acute neurovascular events will have to be carefully titrated so that delayed neuronal, glial and endothelial recovery is not impaired.

**Angiogenesis, neurovascular repair and stroke recovery**

During the acute phase of stroke, the ischemic penumbra suffers milder insults because of residual perfusion from collateral blood vessels compared with the core of the ischemic territory. Over the course of hours to days, the penumbra collapses if therapy is not initiated in time. Besides neuronal death per se, collapse of the acute penumbra can also be viewed in terms of the degradation of cell–cell interactions in the neurovascular unit (Fig. 2). Loss of signaling between astrocytes and endothelium alters tight junction homeostasis and leads to BBB disruption. Perturbations in neuronal–glial signaling lead to loss of proper neurotransmitter dynamics. And loss of matrix–trophic interactions between the vascular and neuronal elements may trigger parenchymal injury beyond ischemia itself. In the face of this acute neurovascular injury, it is beginning to be recognized that evolution of the penumbra may also mediate recovery. The penumbra is not just dying over time. It can also be actively trying to repair itself because endogenous mechanisms of plasticity and remodeling occur over days to weeks after stroke onset [12].

The primary neurovascular responses during stroke recovery are thought to involve angiogenesis and neurogenesis. Angiogenesis is the key step for recovery after ischemia in other organs. So it is reasonable to expect that similar processes would occur in the brain after stroke. In penumbral regions, increased microvessel density has been observed in human patients [40]. In at least one study, the number of new vessels appeared to be related to longer survival times in ischemic stroke patients, suggesting that active angiogenesis may be beneficial [41]. In contrast, older patients who tend to do worse after stroke [42,43] seem to have reduced new vessel formation after stroke [44]. Furthermore, patients who develop dementia after stroke may suffer from reduced blood flow in adjacent cortical regions [45]. This raises the possibility that angiogenesis may improve cerebral perfusion and function as part of a network repair.

The spatial and temporal dynamics of post-stroke angiogenesis are complex and remain incompletely characterized. Nevertheless, it has been generally documented that the proliferation of brain endothelial cells is indeed triggered after ischemic events [44,46]. In mice, endothelial proliferation may begin within a day after ischemia and persist for up to several weeks thereafter [47,48]. Genes correlated with brain angiogenesis have also been extensively assessed in experimental stroke models. For example, endogenous signals for vascular endothelial growth factor (VEGF) appear in both neurons and astrocytes after focal cerebral ischemia [49,50]. Boosting VEGF also seems to promote recovery. Infusing VEGF into the lateral ventricles stimulated angiogenesis and decreased infarct volume in rodent models of focal cerebral ischemia [51]. An increase in angiogenesis by VEGF in rats was associated with reduced neurological deficits after focal cerebral ischemia [50]. In addition to these biochemical and pharmacologic findings, genetic data have also been obtained. In transgenic mice overexpressing human VEGF165, brain microvessel density was significantly elevated compared with wild-type mice before ischemia, and the increase in microvessel density 3 days after stroke onset was improved [52]. These data show that VEGF promotes revasculization after stroke.

Increasing evidence in both human stroke patients and animal stroke models suggests that angiogenesis can occur in the penumbral areas that seek to recover. However, it remains to be fully elucidated whether these new vessels are truly functional. It is worth noting that Lyden and colleagues have proposed a ‘clean-up hypothesis’, whereby newborn vessels serve to facilitate macrophage infiltration, and clear up and remove cellular debris from pan-necrotic tissue [53,54]. This alternate hypothesis would suggest that post-stroke brain angiogenesis is only transient and not permanently involved in neuronal recovery. Nevertheless, the data in aggregate support a beneficial role for angiogenesis and neurovascular repair, together with a close
coupling between angiogenesis and neurogenesis. The reader is referred to more detailed reviews that describe these neurovascular remodeling phenomena [9,55,56]. Here, we now focus on the concept that neurovascular recovery in fact, utilizes the same mediators that appear to underlie acute injury.

As discussed above, a major mediator in typically involved in neurovascular responses is VEGF. In this regard, VEGF is the prototypical biphasic mediator. Like MMPs, VEGF increases BBB permeability in the acute phase in stroke. VEGF administration worsens BBB leakage by ischemic insults. By contrast, VEGF can accelerate angiogenesis and neurogenesis responses in the delayed stroke phase. VEGF can trigger remodeling responses in both endothelial cells and neurons (see Fagan et al. [57] and Hansen et al. [58] for full and detailed reviews for those opposite actions of VEGF). Furthermore, there may also be feedback loops because MMPs can process pro-forms of matrix-bound VEGF into freely diffusible bioactive forms of VEGF [59]. Altogether, the interactions between MMPs and pro-angiogenic mediators such as VEGF should provide a complex but rich substrate for post-stroke angiogenesis.

Neurovascular proteases such as MMPs damage the BBB and cause edema, hemorrhage and neuronal death in the acute stroke phase. However, recent studies suggest that these same proteases may have a beneficial role during neurovascular repair. In a mouse stroke model, peri-infarct cortical areas demonstrate a secondary elevation in MMP-9 in endothelial and glial cells within networks of regrowing microvessels [60]; and inhibition of MMPs during this delayed phase actually made outcomes worse with the development of hemorrhagic and malformed blood vessels and enlarged volumes of infarction and cavitation. Beyond the peri-infarct zone, other brains areas were also involved. Secondary MMP-9 signals co-localized with streams of migrating neuroblasts from the subventricular zone, and inhibition of these MMPs also blocked the movement of these neuroblasts, originally headed towards damaged brain [61].

Beyond VEGF and MMPs, the concept of biphasic neurovascular responses may apply more broadly to a large spectrum of other mediators. The N-methyl-D-aspartate (NMDA) receptor is one of the most intensively studied targets in neuroprotection in acute stroke, because glutamate-induced excitotoxicity has been thought of as the main reason for neuronal cell death. Although NMDA receptor activation in the acute phase leads to neuronal damage, the same NMDA signaling may participate in neurovascular repair (especially neurogenesis) in the recovery phase [62]. In addition to ‘extracellular’ mediators (MMP, VEGF, glutamate activation of NMDA receptors), intracellular signals may also demonstrate biphasic profiles. The stress-activated protein kinase c-Jun N-terminal kinase (JNK) pathway is known to trigger many cell death pathways including caspases, and many studies have shown that JNK inhibitors are neuroprotective in rodent stroke models (see Kuan and Burke [63] for a full and detailed review). However, more recent data clearly support a beneficial role for JNK in CNS disease and repair [64]. JNK signaling is involved in neuronal precursor cell migration, microtubule assembly and axonal guidance during brain development. After injury, this signal can contribute to dendritic sprouting and axonal regrowth. More recently, JNK has also been shown to mediate angiogenesis [65]. JNK mediates the regulation of both VEGF and MMPs, and blockade of JNK cascades with inhibitors can suppress angiogenesis in tumor cell systems [66,67]. Whether similar pathways are activated in cerebral neurovascular repair and remodeling remains to be determined, but given the emphasis on targeting JNK in acute stroke, these types of biphasic repair responses deserve consideration. An uncontrolled wholesale inhibition of JNK may worsen stroke recovery by preventing neurovascular remodeling.

Interactions between angiogenesis and neuronal restoration can also be manifested in terms of circulating EPCs. EPCs are immature endothelial cells which circulate in peripheral blood [68] and are under maturation process to become endothelial cells. Hence, EPCs possess functional and structural characteristics of both stem cells and mature endothelial cells. As discussed above, angiogenesis in the penumbra area is an important natural response to stroke. Although circulating EPCs represent only ~0.01% of cells in the blood under steady-state conditions, EPC numbers are highly affected by stroke onset. Emerging studies are beginning to elucidate the relationship between stroke outcome and the number of circulating EPCs. In rodent models of focal cerebral ischemia, there was a strong correlation between the volume and severity of infarcts and the absolute number of circulating CD34+ and CD133+ cells (both thought to be markers for EPCs) [69]. In clinical stroke patients, an increase in circulating EPCs after acute ischemic stroke was associated with good functional outcome and reduced infarct growth and maturation [70]. Importantly, from flow cytometry measurements, EPC levels were significantly lower in patients with severe neurological impairment compared with patients with less severe impairments at 48 h after ischemic stroke [71]. In mouse cerebral ischemia models, bone marrow-derived EPCs homed to the
ischemic core and participated in cerebral neovascularization [72]. These observations raise the possibility that EPCs can be used as a therapeutic approach for promoting repair (see Rouhl et al. [73] for a full and detailed review). Perhaps, there are even ways to augment EPC function. Recent experiments suggest that high-mobility group box 1 (HMGB1) and interleukin-1beta can promote EPC homing and proliferation, respectively [74,75]; simply increasing motor activity with exercise also seemed to amplify EPC numbers and improve outcomes after focal cerebral ischemia in mice [76]. All these ideas hold promise that combination approaches may be explored to leverage the power of EPCs for angiogenic recovery. However, the precise mechanisms of the EPC contribution to postnatal angiogenesis remain to be elucidated. It has been reported that bone marrow-derived EPCs did not incorporate into the adult growing vasculature [77]. Furthermore, mobilized bone marrow-derived EPCs have been shown to enhance the angiogenic response to hypoxia without differentiation into endothelial cells [78]. These reports suggest that EPCs support angiogenesis indirectly through growth factor release. Therefore, the idea of EPC usage as a clinical application will have to be carefully developed and assessed before EPCs can be safely tested and applied in clinical stroke.

Taken together, accumulating data now suggest that neurovascular mediators span a very wide range of responses after stroke. Some are detrimental, whereas some are beneficial. Perhaps, acute neurovascular responses serve to prepare the substrates required for later angiogenesis and brain recovery (Fig. 3). Because similar signals and substrates are involved, one will have to be very careful in terms of understanding how and when these injury-into-repair transitions take place. Otherwise, acute neurovascular inhibition strategies may interfere with angiogenesis and worsen stroke recovery instead.

Conclusions

The brain is a highly complex organ. Seeking efficient targets to treat brain diseases may be extremely difficult. For stroke, we have seen numerous clinical trials fail. Although there are many reasons why these trials have not worked, the concept of a neurovascular unit has emerged in recent years, to suggest that a broader analysis beyond only neurons is required. Interactions between neuronal, glial and vascular elements in brain mediate function. Loss of proper signaling in the neurovascular unit underlies disease. In this minireview, we briefly overviewed the current knowledge regarding neurovascular injury and repair in stroke. We propose the hypothesis that acute neurovascular events may sometimes represent early triggers for endogenous attempts at delayed angiogenesis later on. Cell–cell signaling in the neurovascular unit is altered, tight junctions are disengaged, extracellular proteases are activated and circulating endothelial precursors may be recruited. Understanding how these acute events transition into delayed neurovascular remodeling is critical. Finding ways to regulate neurovascular perturbations and promote brain angiogenesis may allow us to develop new therapeutic opportunities for stroke.

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