CNS Neuroscience & Therapeutics

EDITORIAL

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Perspective for Stroke and Brain Injury Research: Mechanisms and Potential Therapeutic Targets

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doi: 10.1111/cns.12392

The incidence of stroke has fallen dramatically in Western countries in the past three decades. This resounding success is partially the result of superior control of risk factors such as hypertension, diabetes, and hyperlipidemia. Although many successful experimental results have not translated to clinical application, experimental models have nevertheless made significant contributions to our current understanding of the mechanisms underlying stroke. Thus, stroke prevention and treatment is likely to achieve even more success with further understanding of the molecular mechanisms of ischemic brain injury, the major goal of this special issue.

For several decades, there has been strong experimental and clinical support for the notion that inflammatory and immune responses play critical roles in stroke pathophysiology. One of the major cell types in the brain to control the innate immune response is the microglial cell. First and foremost, microglial activation is thought to be an important defensive reaction against ischemia, as microglia are primarily responsible for phagocytizing toxic cellular debris. However, excessively activated microglia also mediate toxic inflammatory responses by releasing a plethora of harmful substances, such as nitric oxide, reactive oxygen species, matrix metalloproteinase-9, and pro-inflammatory cytokines. These substances can degrade the extracellular matrix and damage the surviving cells at the site of injury. In this issue, Kim, Yenari, and colleagues review the mechanisms underlying microglial activation. Microglial activation is the first step in the inflammatory response of the brain and is often followed by activation of other cell types and the infiltration of neutrophils, macrophages/monocytes, natural killer cells, and T cells from the periphery into the brain. For example, bone marrow-derived macrophages/monocytes are known to migrate into the peri-infarct region of the ischemic brain and to differentiate into microglia. However, it remains controversial whether circulating macrophages/monocytes contribute to the adult microglia pool. Microglia are unique among brain cells in that they can assume a number of polarized phenotypes with beneficial or destructive roles [1]. For example, the M2 microglial phenotype helps to clear necrotic debris and promotes the secretion of anti-inflammatory factors, whereas the M1 phenotype is responsible for propelling pro-inflammatory positive feedback loops.

Although underappreciated for many years, cell-to-cell crosstalk is now known to be essential for the integration of stress responses in the ischemic brain. For example, neurons can modulate microglial activation by a variety of ligand-receptor systems or by releasing trans-modulators such as glutamate, fractalkine, and nitric oxide. Abnormal neuron-to-microglia signaling has been hypothesized to lead to microglial dysfunction in neurodegenerative diseases. Conversely, a reduction in microglial numbers is known to disrupt synaptic function and impair brain connectivity. In other words, neurons and microglia engage in many reciprocal interactions. Microglia can also engage in crosstalk with astrocytes, and both of these cell types are known to become highly activated during cerebral ischemia [2]. However, the underlying processes of activation are quite different. Microglia are activated before astrocytes and may modulate astrocyte activation by the secretion of pro-inflammatory cytokines. Conversely, microglia that are relatively distant from the infarction area can be activated by the transmission of astrocytic Ca^{2+} waves over long distances. Furthermore, astrocytes can inhibit microglial activation by reducing pro-inflammatory cytokines, nitric oxide, and reactive oxygen species. Microglial activation can also negatively or positively affect myelin-producing oligodendrocytes [3]. For example, microglia can promote oligodendrogenesis and improve neurological symptoms. The crosstalk between microglia and oligodendrocytes has mainly been studied in demyelinating diseases and far less is known about it in ischemic stroke. Thus, the significance and molecular mechanisms underlying microglia/astrocyte/oligodendrocyte crosstalk warrant much further exploration.

Cell-to-cell crosstalk is a particularly important feature of the "neurovascular unit," a recent concept that takes into consideration the close interrelationship between neural, glial, and vascular components at the site of injury. The concept of the neurovascular unit has prompted a paradigm shift away from our previously neurocentric perspective of the ischemic brain. In this issue, Lok, Wang, and colleagues make the argument that saving only the neurons will be insufficient for full restoration of tissue integrity and functional outcomes. The authors review the concept of the neurovascular unit as it applies to brain trauma. To some degree, the brain may attempt to recover from stroke and traumatic brain injury by initiating angiogenesis at the site of ischemic injury. However, cell–cell signaling between the components of the neurovascular unit is heavily disrupted in stroke and trauma, thereby increasing cell death at the site of injury. Thus, the therapeutic potential of vascular remodeling after acute injuries has also been discussed in the literature [4–6]. Expansion of the neurovascular concept into fields such as traumatic brain injury is an important move because there are no satisfactory therapies for victims of acute brain trauma. However, Shi, Gao, Chen, and colleagues have shown in this issue that ethyl pyruvate attenuates the breakdown of the blood–brain barrier in

experimental traumatic brain injury, perhaps by reducing inflammatory markers such as matrix metalloproteinase 9 and mitigating microglial activation. Ethyl pyruvate was thereby able to confer long-lasting neuroprotection against neurological deficits, a finding that holds promise for the future treatment of trauma victims. In general, increasing numbers of stroke studies such as this are examining animals at long (~1 month) intervals following cerebral artery occlusion to ensure that the protection is not fleeting.

Cell-to-cell communication also occurs over surprisingly long distances, such as between the brain and peripheral organs. For example, the injured brain releases alarm signals that reach organs as far away as the spleen and lead to the entry of spleen-derived cells such as neutrophils and monocytes/macrophages into the brain. In this issue, Liu, Hu, and colleagues discuss therapeutic strategies aimed at splenic responses to ischemic stroke. Rather intriguingly, the size of the spleen is known to change dramatically after stroke in humans, with an early contraction followed by a subsequent expansion. In experimental models of stroke, this early shrinkage of the spleen is correlated with the extent of ischemic damage. Whether these splenic responses are beneficial or injurious may depend on the severity of the ischemic injury and on the time that has passed since stroke onset. The variability in the effects of splenectomies on infarct size in the literature is likely to reflect the dual-faced nature of the inflammatory response itself. The orchestration of crosstalk or dialog between the brain and the peripheral immune system after stroke injury has been recently reviewed elsewhere [7].

In this issue, Kim, Cho, and colleagues report that inhibition of CD36-mediated inflammation significantly diminishes acute ischemic injury after transient, but not permanent stroke. The authors use CD36-deficient mice and SS-31, a cell-permeable peptide that downregulates CD36 pathways. Both techniques attenuate the stroke-induced increase in monocyte chemoattractant protein and its chemokine receptor, CCR2. These results help confirm and extend the importance of inflammation in stroke pathophysiology.

Another emerging topic in stroke research is neurogenesis, the discovery of which raised the hope in many scientists that we will be able to replace the neural cells that have been lost to ischemia [8]. In addition to replacing lost cells, stem cells also have the potential to release trophic factors to protect the remaining survivors [9]. As a result, undifferentiated stem cells have been extensively tested and evaluated for their therapeutic potential in ischemic stroke. In this issue, Tang, Yang, and colleagues describe the many opportunities and challenges faced by stem cell researchers. The majority of stem cell studies have explored the efficacy of transplanting a single type of stem cell. However, a combination of two types of stem cells has recently been explored to improve the efficacy of this therapy and may boost the survival of stem cells and their migration into the lesion area. Currently, there are more than 70 stem cell studies with known status on the clinicaltrials.gov website. About half of these studies have passed the phase I safety evaluation and entered the phase II efficacy test. However, several challenges remain to be overcome before stem cell therapy can be translated to stroke victims. These challenges include increasing transplanted stem cell survival, improving stem cell migration, proliferation, and differentiation in the injured area. Indeed, there remains considerable controversy regarding the fate of stem cells after transplantation. Although intravenous and intraarterial injections of stem cells improve ischemic stroke recovery, little is known about where these cells migrate and what they differentiate into. Dynamically tracking transplanted cells and assessing their bioavailability and distribution using novel whole-body live imaging systems would help shed more light on the underlying mechanisms. The potential for cell transplantation-based therapies has also been reviewed recently [10,11].

In this issue, Pabon, Borlongan, and their colleagues hypothesize that gender plays a key role in determining the "stemness" of adult stem cells. Gender has long been established as a risk factor for developing stroke. For example, males have a higher risk for stroke at younger ages, a trend that is reversed after women enter menopause. Sex-specific stroke outcomes are thought to be regulated in part by microRNAs that target stroke-related genes. Thus, in this issue, Kim and Vemuganti review the effects of gender and age on stroke outcomes and the role of steroid hormones, cell death pathways, and microRNAs.

Intracerebral hemorrhage accounts for a significant fraction of stroke incidents and is often fatal. Furthermore, those who manage to survive often suffer from catastrophic cognitive and sensorimotor deficits. Finding effective treatments is therefore a matter of some urgency to stroke and hemorrhage researchers. The serious issues facing researchers who study intracerebral hemorrhage have been outlined [12]. In this issue, Zhao, Aronowski, and colleagues examine the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) as instrumental in anti-inflammatory and antioxidant defense. PPARγ agonists are therefore being examined as potential therapies for both stroke and intracerebral hemorrhage. The interactions of PPARγ with the NFκB and Nrf2 proteins, which are involved in inflammatory responses or antioxidant defense, may also deeply influence stroke outcomes. The risk for intracerebral hemorrhage is an important consideration when using tissue plasminogen activator (tPA), the only FDA-approved treatment for ischemic stroke. Unfortunately, tPA must be administered only within a limited time after stroke onset. The use of tPA is known to be a double-edged sword; levels must be titrated carefully and the timing of delivery is critical. In this issue, Yang and Kuan describe how low levels of tPA are neuroprotective, whereas high levels exacerbate injury and review evidence that natural induction of endogenous tPA in the brain plays a detrimental role in neonatal hypoxia–ischemia, a condition that can leave infants with long-term neurological deficits. Yang and Kuan report that an inhibitor of tPA known as CPA1 protects against hypoxic–ischemic injury both in the absence and in the presence of inflammation. The intranasal approach to delivering this molecule into the brain holds significant promise for clinical translation.

Other treatment strategies that are being carefully considered by stroke researchers include preconditioning strategies, recently reviewed by Stetler and colleagues [13]. Preconditioning is the phenomenon whereby mild, sublethal stress induces a compensatory homeostatic reaction that prepares cells and tissues to battle subsequent injuries. In other words, transient ischemic attacks can protect against subsequent ischemic episodes of longer duration [14]. This strategy has even been applied following the onset of stroke, in which case it is known as postconditioning. The advantage of pre- and postconditioning strategies is that

endogenous protective mechanisms are boosted in the absence of any pharmacological intervention. Another advantage is that the researcher can identify evolutionarily conserved defense mechanisms that cells have developed all on their own. Additional strategies being considered by stroke researchers include the use of volatile gases such as isoflurane, which have been shown to exert neuroprotective effects and are already in clinical use for anesthesia [15,16]. Other authors have also pointed to interventions such as hypothermia as alternative and complementary strategies [17,18].

In summary, many exciting new treatments lie on the horizon and the contents of this issue present a variety of strategies to combat the sequelae of ischemic injury. While there is hope for eventual success, it is also obvious that much work still needs to be done. Indeed, the lack of clinical translation of therapies that show promise in animal models has been an ongoing source of frustration and criticism in the stroke field. When such frustrations arise, it is tempting to conclude that our singular focus on rodent models has translated into protecting only mice and rats from injury, not actually protecting any humans. However, it is important to bear in mind that the success of tPA owes much to research on animal models. As mentioned above, some of our failures may also reflect our tendency to only measure neuronal survival and not the survival of glia and vascular components. Another important measurement to include when assessing protection is to ensure that new therapies also reduce markers of inflammation. In other words, our measurements of "protection" need to become much more multifaceted and encompass more cell types. Studies on larger organisms, such as pigs and nonhuman primates, will also be essential for accelerating drug discovery. It is important to include animal models with a more complex brain and a glia-to-neuron ratio that is closer to that found in humans. In conclusion, the emerging emphasis of stroke research on glia and the neurovascular unit will pay dividends in the long run and has already contributed significantly to our current understanding of stroke pathophysiology.

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