Microglia in the Pathophysiology of Hemorrhagic Stroke and the Relationship Between Microglia and Pain After Stroke: A Narrative Review

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ABSTRACT

Stroke is a leading cause of death worldwide, and about a quarter of stroke patients are dead within 1 month. The prognosis is even worse for those with hemorrhagic stroke because the 1-month mortality approaches 50%. Besides, most patients who survive experience complications such as nausea, vomiting, and chronic pain. These adverse experiences, especially the existence of chronic pain, can lead to a decline in the patient’s quality of life. In order to improve the treatment and prognosis of hemorrhagic stroke, there is an urgent need to understand its pathophysiological mechanism as well as the chronic pain it induces. This paper reviews studies of the molecular mechanisms of hemorrhagic stroke, especially the activation of microglia and the relationship between microglia and pain after stroke, which could shed new light on hemorrhagic stroke treatment.

Keywords: Microglia; Hemorrhagic stroke; Pain

Key Summary Points

- Chronic pain is one of the most troublesome sequelae of stroke. It is reported that about 11–55% of stroke patients will suffer chronic pain.
- Pain after stroke is related to the activation of microglia; there is an urgent need to understand the associated pathophysiological mechanism.
- This paper reviews studies of the molecular mechanisms of hemorrhagic stroke (HS), especially the activation of microglia and the relationship between microglia and pain after stroke.
- Microglia are a therapeutic target for hemorrhagic stroke and HS-related pain.

INTRODUCTION

Hemorrhagic stroke (HS) has the second highest incidence of any type of acute stroke, accounting for 10–20% of all strokes [1]. Every year, more than a million people around the world are affected by this disease, and this number will...
increase greatly due to the aging of populations. Hemorrhagic stroke can induce vomiting, dizziness, impaired consciousness, and even hemiplegia. In addition to the abovementioned acute symptoms, most patients will suffer paraesthesia, in which the most unbearable feeling is chronic pain, which affects the prognosis and quality of life of those patients.

Pain is one of the most troublesome sequelae of stroke. It is reported that about 11–55% of stroke patients suffer chronic pain, which is mostly related to anxiety caused by long-term illness [2, 3]. The most common manifestations of chronic pain after stroke are shoulder pain, central post-stroke pain, cramps, and tension-type headaches [4]. The pathogenesis of post-stroke pain (PSP) remains unknown, but it has been suggested that the underlying causes include hyperecxcitation in the damaged sensory pathways, central nervous system lesions, or a combination of the two. For central nervous system lesions, the most important factor is microglia dysregulation.

In this review, we provide an overview of microglial function after hemorrhagic stroke and in the process of PSP, especially microglial polarization, modulators, and interactions with other cells. We also address clinical observations and highlight new therapeutic directions relating to microglia for hemorrhagic stroke and PSP.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

ACTIVATION OF MICROGLIA FOLLOWING HEMORRHAGIC STROKE

When hemorrhage occurs, brain damage, which is mainly caused by edema or hematoma, will develop rapidly within the first few hours. Brain edema and hematoma increase the intracranial pressure sharply, which induces herniation and death [5]. Later on, microglia act as intracranial phagocytes, protecting the brain tissue by eliminating blood cells and other small molecules. In the middle and late stages, microglia transform into inflammatory cells and secrete large amounts of proinflammatory factors to destroy the vascular endothelium. The direct phagocytosis and the indirect effects of proinflammatory factors will aggravate the hemorrhagic injury. Activation and modulation of the microglial phenotype not only help to absorb the hematoma or edema but they also improve white matter integrity, brain repair, and functional recovery [6].

MOLECULAR MECHANISMS UNDERLYING MICROGLIAL ACTIVATION FOLLOWING HEMORRHAGIC STROKE

Phenotypic Polarization of Microglia and Markers

In response to CNS inflammation and brain injury, microglia cells can be activated; they then migrate to the lesion and envelop the injury zone. Activated microglia have been found in the perihematomal region as early as 1 h after hemorrhagic stroke [7], where they provide protective effects—clearing the debris and hematoma and promoting neurogenesis. Microglia acutely stimulated by a hemorrhage can be activated to two polarization states: M1, for promoting inflammation, and M2, for suppressing inflammation. A few minutes after the stroke, M1-phenotype microglia cells secrete and recruit inflammation factors such as Toll-like receptor 2 (TLR2) and TLR4 [8–10], INF-γ, and complement C3a and C5a. Almost the proinflammation factors are induced by the M1 type. The proinflammatory molecules activated by M1 microglia comprise the interleukin (IL) family (IL-1β, IL-6, IL-12, and IL-23), tumor necrosis factor alpha (TNF-α), chemokines (CX3C), redox molecules (NADPH oxidase, phagocyte oxidase, and inducible NO synthase), costimulatory proteins (CD40), and major histocompatibility complex II (MHC-II) [11–15].

On the contrary, M2 microglia play a protective role by promoting anti-inflammatory effects and suppressing oxidative injury [16, 17]. These cells create anti-inflammatory mediators...
such as IL-10, transforming growth factor beta (TGFβ), glucocorticoids, arginase-1, Ym-1 (chitinase 3-like 3), and CD206 [18–20]. M2 microglia are also considered to be nerve-repairing cells, as they secrete anti-inflammatory factors and upregulate neuroprotective factors in CNS disease.

M1 and M2 microglia express different factors, so different molecular markers can be used to distinguish these two phenotypes by double or triple immunofluorescence. Usually, M1 microglia markers can be labeled with MHC-II, CD16, CD32, CD80 (B7-1), CD86 (B7-2), and CD40 (TNFR). M2-cell-specific antigens include Ym-1, CD206 (mannose receptor), CD68, and arginase-1.

Dynamic Changes in Microglial Polarization

It was observed that hemorrhage induced the activation of microglia with high M1 phenotype gene expression at 24 h, while the expression of M2 phenotype genes gradually increased in the first 2 weeks, and the expression time varied slightly in different brain regions [21]. This contrasts with what is seen following ischemic stroke, where the M2 phenotype increases first, followed by the M1 phenotype [22]. These results suggest that the spatiotemporal dynamics of microglia polarization may be related to the degree of damage and the disease model considered.

Based on existing evidence and studies, once microglia have been activated by acute injury, they can change dynamically; this is especially seen in collagenase-induced [23, 24] and blood-induced [25] models. In mice with collagenase-induced cerebral hemorrhage, the microglia complete the transition from M1 to M2 phenotype within 1–3 days. In mice with blood-induced cerebral hemorrhage, this transition occurs within 1 week. Generally speaking, about 3 days after an intracerebral hemorrhage, microglia polarize into the M1-like phenotype, which is the main reason for the activation of microglia in the acute phase after hemorrhage, whereas M2-like microglia may play a role in the long-term recovery from stroke. Furthermore, studies have shown that in the process of microglial polarization, M2-type cells have multiple subtypes that are involved in damage repair and anti-inflammatory processes, and they play an important role (see Table 1 [26]).

Other Important Factors Related to Microglia Activation

Microglia are activated in the core part of the hemorrhagic stroke lesion and the perihe-matoma [23, 24]. The activated microglia produce a large number of inflammatory mediators that cause the activation of and changes in various inflammatory signal pathways [27, 28].

NLRP3

The collagenase-induced hemorrhagic stroke model presented an activated NLRP3 (NACHT leucine-rich repeat protein 3) signal pathway. Typically, NLRP3 activation is induced by the inflammatory response caused by bleeding; heme and other blood components in the blood can activate this pathway [29]. In addition, endogenous cytokines and other stimuli (pathogens, etc.) can activate the classical NLRP pathway. Exogenous or endogenous stimuli act through TLR4 or TNFR to activate the NF-κB pathway together with caspase-8 and FADD (FAS-associated death domain protein) [30], and they also initiate the transcription and translation of NLRP3 and pro-IL-1β. After NLRP3 has been activated, lysosomes are destroyed, resulting in the release of cathepsin B. At the same time, active caspase-1 can cleave the precursor IL-1β and the precursor IL-18 to mature [31]. In the nonclassical pathway, LPS directly induces the activation of intracellular caspase-11 (homologous to human caspase-4 and -5) [32], which in turn activates NLRP3 [32].

TREM2

Triggering receptor expressed on myeloid cells 2 (TREM2) is a natural immune receptor that is mainly expressed in myeloid cells. In the nervous system, it is mostly expressed in microglia, with a small amount expressed in astrocytes and neurons [33, 34]. After injury, its protein
expression density in microglia is about 300 times that of other glial cells [35, 36]. This type of receptor is related to a variety of cellular processes, such as cell proliferation, regulating the release of proinflammatory factors, and promoting the phagocytosis of microglia [37]. Unlike TREM1, TREM2 is considered to provide negative feedback regulation [38]. This effect has been confirmed in a variety of neurological disease models. For example, in Alzheimer's disease and multiple sclerosis models, it was found that overexpression of TREM2 can inhibit the inflammatory response and play a protective role [39–41]. The latest research has shown that the endogenous expression of TREM2 in the ipsilateral/right hemisphere after intracerebral hemorrhage increases in a time-dependent manner, reaching a peak at 24 h after intracerebral hemorrhage [42]. The PI3K/Akt signaling pathway serves as a downstream target of TREM2 [43, 44], which is likely to play a vital role in the process of cerebral hemorrhage. Therefore, the research and development of drugs that can induce high expression of TREM2 have become a broad prospect for clinical therapeutic applications.

**VAP1**

Vascular adhesion protein 1 (VAP1) is a glycated protein that can regulate inflammation and at the same time provide a molecular basis for the adhesion and migration of white blood cells [45]. A study of subarachnoid hemorrhage in rats found that the use of VAP1 inhibitors can reduce the deterioration of neurological function after injury [46]. Similarly, VAP-1 inhibitors can downregulate the expression of adhesion molecules, thereby reducing the infiltration of neutrophils into the injured site. Subsequently, the proinflammatory cytokines TNF-α and MCP (monocyte chemoattractant protein) were significantly reduced, suppressing the activation of microglia or macrophages and ultimately improving the behavioral neurological function and brain edema of mice [47].

**Microglial Crosstalk with Other Cells During Hemorrhagic Stroke**

**Astrocytes**

The main role of astrocytes is to connect neurons to the CNS vasculature and act as a bridge. Anatomically speaking, the unique structure of an astrocyte extends from the cell body of the astrocyte to its end-feet, which attach to the basement membranes of endothelial cells. This structure helps maintain the integrity of the blood–brain barrier and prevents harmful substances in the blood from entering the brain. However, in central nervous system inflammation, microglia receive chemical stimulation and migrate to the blood–brain barrier. In this process, microglia initially play a protective role by secreting endothelial connexin (claudin-5); later, as the inflammation continues, they swallow the ends of astrocytes, breaking the blood–brain barrier and aggravating the inflammation of the nervous system [48–50]. More interestingly, it was observed that astrocytes could transform into mature neurons.
in situ under stroke conditions [51], suggesting that the mature brain is capable of replacing neurons lost after injury [52], even though these newborn neurons from astrocytes have a little function which cortical neurons have.

**Monocyte Macrophages**

Similar to microglia, these are innate immune cells and respond earliest to lesions. Interferon γ (IFN-γ) secreted by TH1 cells can convert macrophages into activated histiocytes, which can produce nitrous oxide and other oxygen-derived free radicals to kill pathogens. However, excessive release of inflammatory factors can exacerbate cell damage in hemorrhagic stroke. Macrophages have the same activation pathways and effects as microglia during the hemorrhage process. The markers of these two kinds of cells are almost the same, e.g., IBA1, CD11b, and F4/80 [53]. However, there are at least two differences between microglia and monocyte macrophages. Firstly, some studies have shown that macrophages that are activated by injury and migrate from the blood into the lesion express even more of the M2 phenotype than microglia that proliferate in situ. Macrophages that infiltrate from blood vessels have an important phagocytic function and inflammatory properties, while in situ microglia seem to preferentially remove cell debris [54]. In addition, infiltrating macrophages may be cytotoxic regardless of whether they adopt the M1 or M2 phenotype [55]. These results indicate that although the expression of M2-type macrophages increases, M2 microglia have more advantages than M2-type macrophages in brain protection. Second, the marker CD45lowCD11b+ was found to be expressed in microglia but not in macrophages. Similarly, TMEM119 is only expressed in macrophages [56]. Accordingly, the above two markers can be used to distinguish microglia and macrophages.

Different causes and environments of injuries may lead to slight differences in their effects. As we all know, the brain has the ability to self-repair after it has been damaged [57]. In this process, microglia and macrophages work together to surround the injured area and secrete various nutritional factors so that the protruding formation of new granular cells will follow the nutritional gradients toward the injured area, ensuring that the correct shape is formed. Microglia and macrophages participate in the whole process until the synapse is completely formed [56, 58].

**MICROGLIAL ACTIVATION PLAYS AN IMPORTANT ROLE IN PAIN POST HEMORRHAGIC STROKE**

The activation of microglia is closely related to the occurrence of pain after stroke. Microglia are activated in the core part of the hemorrhagic stroke lesion and the perihematoma [23, 24]. The activated microglia produce a large number of inflammatory mediators that activate and change various inflammatory signal pathways [27, 28]. Inflammation is considered the main pathological feature that causes pain. The activation of the P2X4/P2RX7 receptors in microglia [59–61] and the activation of NLRP3 in microglia are both related to the occurrence of pain after cerebral hemorrhage and stroke [62, 63]. In the thalamic hemorrhage model, depletion of microglia can effectively prevent the development of analgesia [64].

Nagaka et al., using a macaque model [65], found that microglial activation may play a key role in post-stroke pain, although the associated mechanism remains unclear. At present, it is mainly believed that the increased secretion of chemokines and proinflammatory factors after the nervous system is damaged is the main cause of pain symptoms. Chemokines are small molecular proteins that are often divided into four subtypes: CC, CXC, XC, and CX3C. The CX3C family currently only includes one member: CX3CL1. When damage occurs, the chemokine CX3CL1 is expressed in primary afferent and spinal neurons and induces microglial activation through its microglia receptor, CX3CR1 [66, 67]. CX3CL1 plays an important role in the development of neuropathic pain. In the neuropathic pain model induced by paclitaxel, the expression of the CX3CL1 protein in spinal cord neurons can be upregulated through the NF-κB-dependent H4 acetylation mechanism [68]. When CX3CL1 inhibitor is injected intrathecally, it can reverse
the mechanical hyperalgesia and thermal sensitivity induced by cathepsin S [69]. When neuropathic pain occurs, the activation of and functional changes to microglia play an important role [70]. During the transition from immature to mature microglia, the macrophage transcription factor MAFB is greatly upregulated [71]. At the same time, mice that selectively knock out the Mafb gene in microglia do not develop hyperalgesia, which confirms that MafB plays an important role in the process of neural pain [72].

There are many other mechanisms for post-stroke pain, but they are not well understood, and mainly include mitochondrial Ca\(^{2+}\) uptake, NLRP3 activation, and endoplasmic reticulum stress. Most of these are caused by the massive release of proinflammatory factors to the pain-causing effect [62, 73, 74].

As mentioned above, although the activation of microglia plays an important role in post-stroke pain, no definitive studies have identified the type of microglia that plays a major role in PSP. Moreover, the specific mechanism of M1 and M2 microglia activation in post-stroke pain is not clear. Of course, this also provides possibilities for future research directions (Fig. 1).

**MICROGLIA AS A THERAPEUTIC TARGET FOR HEMORRHAGIC STROKE AND HS-RELATED PAIN**

At present, the clinical treatment of hemorrhagic stroke is mostly based on the cause of treatment. Conventional treatments include hemostasis, lowering the intracranial pressure, preventing complications, and the surgical removal of intracranial hematoma. This shortens the procession of the disease to some extent, and reduces the direct stimulation of physical damage. Thus, when the primary cause is controlled, the continuous damage from inflammation will be greatly reduced.

In recent years, more and more studies have indicated that the interactions of numerous neuroimmune cells in the central nervous system play an essential role in neuropathic pain. Neuropathic pain is not only related to the activation of neurons but also to the response of immune cells [75]. Previous studies have shown that the activation of immune cells, particularly glial cells (including microglia), is involved in neuropathic pain. Glial cells actively communicate with neurons through direct connections, synapses, and the release of neuroregulatory chemicals that affect neuronal firing and signal transmission within the cell [76]. Under neuropathological conditions, microglia express a wide range of neurotransmitter receptors, which leads to intracellular signal transduction (e.g., STATs, NF-κB, and MAPKs) and to a series of cascades that are involved in the development of neuropathic pain. Analgesics targeting the microglial pathway have achieved a certain level of clinical efficacy.

As they are the brain’s innate immune cells, microglia can not only differentiate into different subtypes to cope with the development of disease during hemorrhagic stroke, but they can also secrete nutrients to induce neuronal axons to transform damaged regions for regeneration. To a certain extent, the protective effect of M2-type microglia is enhanced while the proinflammatory effect of M1 is weakened. M2 microglia exert their protective effect through the secretion of remodeling factors such as VEGF, BDNF, and matrix metalloprotease-9 [77, 78]. Therefore, based on the aforementioned effects of microglia, microglia-based therapy and any therapeutic strategy aimed at the regulation of these immune cells may prove to be promising and practical therapeutic methods.

This cell therapy strategy can be divided into two main aspects: the inhibition of M1 proinflammatory type cells and factors or receptors—for example, anti-HMGB1 antibody treatment attenuates the brain injury after stroke [79] and anti-inflammatory cytokines (such as IL-4, IL-10, and TGF-β) can improve the brain recovery process after injury [80]—and the promotion of M2 protective-type cells. Interestingly, activated protective cytokines, such as transforming growth factor-β (TGF-β) and IL-10, may suppress inflammation and promote axonal growth and angiogenesis [7, 81].
Multiple forms of microglial activation and associated inflammatory responses are being considered as new targets for the development of effective treatments for neuropathies. As previously discussed above, the activation pattern of microglia varies greatly during neuropathic pain, with microglial activation peaking in the first few days after injury and declining from 3 days to 2 weeks after injury. Further research is needed on the potential mechanisms through which microglia are involved in neuropathic pain and their role in neuropathic pain during the hemorrhagic stroke recovery phase.

CONCLUSION AND PERSPECTIVES

A large number of studies have proved that the activation of microglia plays an important role in the pathophysiological progression of ICH. Different cell phenotypes may contribute differently to neuroinflammation in brain disease models. Regulating the function of microglia can reduce hemorrhage-related brain damage and promote tissue repair and functional recovery. However, given that microglia are widespread in the central nervous system, do microglia in the brain and microglia in the spinal cord play the same role in PSP after intracerebral hemorrhage? And does the regulation of microglia function during certain periods and for certain phenotypes improve pathological pain after stroke? This is not yet clear. Simultaneously, studies have shown that the underlying mechanism may vary with pain phenotype. Therefore, the specific role of microglia in post-stroke pain after intracerebral hemorrhage needs to be further studied.

There are still many limitations of existing animal models of cerebral hemorrhage.
Although research into the cellular and molecular mechanism of pathological pain in stroke has become more and more prominent, most studies have failed to make the leap from basic experiment to clinical application. This also highlights the importance of establishing a clinical stroke model. Progress in science and technology is expected to lead to a greater understanding of the role of microglia in pathological neuralgia after intracerebral hemorrhage, and thus to improve our understanding of the mechanism of pathological pain after stroke, allowing the development of an effective treatment for post-stroke pathological neuralgia [82–87].

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