



Role of inflammasomes in neuroinflammation after ischemic stroke

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Ischemic stroke is a devastating disease for which there is no effective medical treatment. In the era of extensive reperfusion strategies, established neuroprotectant candidates and novel therapeutic drugs with better targets are promising for treatment of acute ischemic stroke. Such targets include the inflammasome pathway, which contributes significantly to the pathogenesis of ischemic stroke. Following ischemic stroke, damage-associated molecular patterns from damaged cells activate inflammasomes, incur inflammatory responses, and induce cell death. Therefore, inhibiting inflammasome pathways has great promise for treatment of ischemic stroke. However, the efficacy and safety of inflammasome inhibitors remain controversial, and better upstream targets are needed for effective modulation. Herein, the roles of the inflammasome in ischemic injury caused by stroke are reviewed and the potential of neuroprotectants targeting the inflammasome is discussed.

Keywords: Stroke, Inflammasomes, Inflammation, Ischemia

Introduction

Stroke is a leading cause of permanent disability and death, causing a significant burden to the rapidly aging society [1]. Stroke occurs through a sudden compromise of cerebral blood flow, which either results from occlusion or rupture of cerebral vessels. The ischemic or hemorrhagic brain receives an insufficient supply of oxygen and glucose, leading to failure of cell respiration and cell membrane rupture. Because the regenerating capacity of injured brain cells is limited, a preventive strategy is crucial to improve the outcomes before irreversible damage occurs in individuals who have had a stroke. Currently, effective medical therapies for acute ischemic stroke include tissue plasminogen activator (tPA) and neuroprotectants. The clinical use of tPA is limited due to the narrow therapeutic window, hemorrhagic side effects, and reperfusion injury. Neuroprotectants are anticipated to fill this gap; however, no drug has proven efficacious. Conventional neuroprotectants aim to prevent neuronal

death by intervening in intracellular or extracellular signals without consideration of reperfusion at occluded vessels [2]. In the absence of reperfusion, the effectiveness of neuroprotectants might be limited. Due to increasing opportunities for reperfusion with endovascular thrombectomy, clinicians can repurpose neuroprotectants with restoration of cerebral blood flow.

Inflammatory processes occur during the course of cerebral ischemia shortly after occlusion to the regenerative phase [3]. The inflammatory processes are considered sterile inflammation because they are augmented by damage-associated molecular patterns (DAMPs) rather than by pathogen-associated molecular patterns [4,5]. Sterile inflammation caused by DAMPs is thought to play crucial roles in the pathogenesis of ischemic stroke and might be a significant therapeutic target to protect the brain from stroke-induced damage [6]. Inflammasome-mediated inflammation is a key mediator of sterile inflammation

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after ischemic stroke [7,8]. The strategy for inflammasome inhibition is very promising but needs further research regarding the best scenarios for clinical application. Herein, the roles of the inflammasome in ischemic stroke are reviewed and potential values and challenges of therapeutics targeting inflammasomes in ischemic stroke are discussed.

Inflammation in the pathogenesis of ischemic stroke

Ischemic brain injury and inflammation

In the ischemic condition, brain cells undergo a complex cascade of cellular and molecular changes, including excitotoxicity, oxidative stress, inflammation, and apoptosis, which lead to irreversible damage. Neurons are more vulnerable to ischemia than are other brain cells [9]. The pathophysiological responses to ischemia trigger one another in a positive feedback loop, leading to innate immunity-induced sterile inflammation that further promotes tissue damage during the acute phase [10]. Following cerebral ischemia, endothelial activation increases blood-brain barrier (BBB) permeability and activation of inflammatory mediators, all which recruit peripheral inflammatory cells to the site of damage [11]. Infiltrating neutrophils and macrophages release inflammatory cytokines, compromise blood supply, and exacerbate brain damage [12]. Microglial activation and astrocyte proliferation further boost inflammatory responses and regulate the viability of neurons [13,14]. Furthermore, the inflammatory response is not contained within the ischemic core but is also observed around the peri-infarct region. This surrounding area is called the inflammatory penumbra, which might be a critical target for therapeutic intervention [15].

Sterile inflammation pathway

Sterile inflammation is mediated by various neural cell types and numerous receptors and downstream signaling molecules. Ischemic neurons release DAMPs, which initiate sterile inflammation by binding to pattern recognition receptors (PRRs) on inflammatory cells. DAMPs include adenosine triphosphate (ATP), chromatin-associated protein high mobility group box 1, heat shock proteins, and uric acid. PRRs are classified into transmembrane proteins such as toll-like receptors (TLRs) and C-type lectin receptors, cytoplasmic proteins such as retinoic acid-inducible gene-I-like receptors (RAGE) and nucleotide-binding oligomerization domain-like receptors (NLRs), and DNA sensors localized in the cytoplasm and nucleus [16]. PRRs are expressed abundantly by astrocytes, microglia, neutrophils, and macrophages [17]. The DAMP-PRR interaction trig-

gers production of tumor necrosis factor- α , interleukin-1 (IL-1), and IL-18 [18]. Microglia express high levels of PRRs, which mediate proinflammatory signals activated by neurotoxic substances such as DAMPs. Proinflammatory cytokines recruit peripheral inflammatory cells, activate neighboring microglia, and exacerbate secondary tissue damage [19].

Pyroptosis

Inflammatory programmed cell death is termed pyroptosis, which is a key process in the pathology of ischemic stroke [20]. DAMPs stimulate inflammasomes, which are intracellular PRRs, and mediate various downstream events such as maturation of IL-1 and IL-18, activation of caspase-1 or caspase-4/5/11, and activation of the effector protein gasdermin D (GSDMD) [21]. Activated GSDMD creates pores in the cell membrane, and subsequent swelling leads to lytic cell death and release of cytokines and other cellular DAMPs [22]. Inflammasomes are activated in neurons and astrocytes shortly after ischemic stroke and later significantly in microglia [7]. Pyroptosis is a direct pathway for ischemic neuronal death and releases various proinflammatory cytokines into the extracellular space, which exacerbates neuronal death [23]. Modulating pyroptosis after ischemic stroke can provide a new avenue for treatment of stroke.

Structure and activation of the inflammasome

The inflammasome was first described in 2002 as a central mediator of the innate immune response to tissue injury [24]. DAMPs trigger innate immune responses through various types of PRRs present on the cell membrane or within cells. Inflammasomes are large, macromolecular, receptor-like structures that act as a type of intracytoplasmic PRR [17]. The inflammasome complex comprises three domains; cytoplasmic PRR, adaptor protein, and effector protein [25].

Intracytoplasmic PRRs include the NLR family and the pyrin and HIN domain-containing (PYHIN) family. In the NLR family, there are NLR pyrin domains containing 1 (NLRP1) and 3 (NLRP3), NLR family apoptosis inhibitory proteins, and NLR caspase activation and recruitment domain (CARD) containing 4 (NLRC4); in the PYHIN family, the cytoplasmic PRR is absent in melanoma 2 (AIM2) [26,27]. The adaptor protein is apoptosis-associated speck-like protein containing CARD (ASC), and the effector protein is the inactive precursor of caspase-1 [25]. The NLR family has three domains, which include the C-terminal containing leucine-rich repeats (LRRs), central NACHT, and N-terminal pyrin domain (PYD) [28]. The C-terminal domain is involved in ligand sensing and stably interacts with hydropho-

bic molecules, such as DAMPs. The central domain is implicated in oligomerization and assembly of inflammasome structures. The N-terminal domain supports PYD/PYD interactions with ASC, and the attached ASC recruits procaspase-1 and generates active caspase-1 [29]. Different types of inflammasomes sense DAMPs via specific or interactive activities, allowing detection of distinct molecular patterns of various tissue injuries.

Inflammasomes are activated by two processing steps (Figure 1). The first step is the priming phase that activates nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Because the basal level of inflammasome is too low to respond to ligands [30], an initial priming signal is required to increase transcription and translation of inflammasomal components. NF-κB and MAPK upregulate the expression of inflammasome components. The second step is the activation phase, which initiates the assembly of the inflammasome structure. During this step, inflammasomes initiate homo- or hetero-oligomerization and recruit adaptor and effector proteins. Bruton's tyrosine kinase (BTK) induces ASC phosphorylation and facilitates activation of the NLRP3 inflam-

masome [31]. An activated inflammasome complex induces cleavage of procaspase-1, which leads to maturation of pro-IL-1 and pro-IL-18. Release of caspase-1, IL-1, and IL-18 initiates the cascade of inflammatory programmed cell death that is pyroptosis (Figure 1). During pyroptosis, inflammasomes are released into the extracellular space and further amplify and prolong the inflammatory response, ultimately leading to cell death.

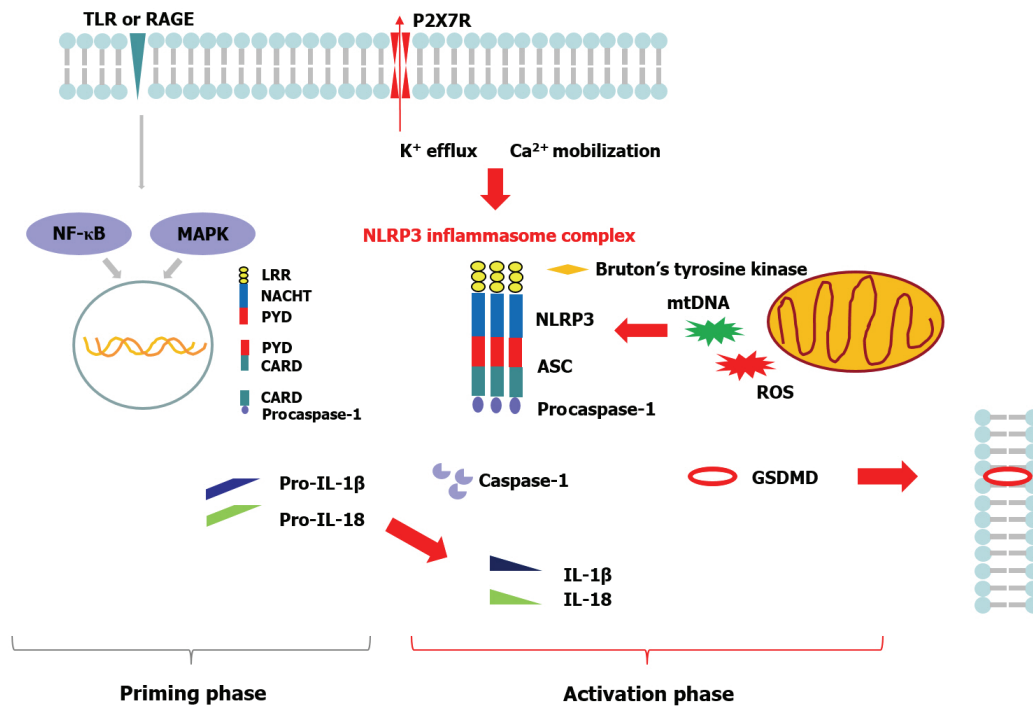
Involvement of inflammasomes in stroke pathophysiology

Inflammasomes, which are abundantly expressed in brain cells, recognize brain injury and modulate inflammatory responses in an injury-specific manner. Ample evidence has shown involvement of inflammasomes in the pathogenesis of stroke [7,32-34]. Specifically, four inflammasomes, NLRP3, NLRP1, AIM2, and NLRC4, have been investigated in various stroke models.

NLRP3

In the pathogenesis of ischemic stroke, NLRP3 has been the most widely investigated inflammasome. The NLRP3 level is

Figure 1 Inflammasome structure and activation process



Intracellular signaling processes for action of inflammasome complex include priming and activating phases. TLR, toll-like receptor; RAGE, retinoic acid-inducible gene-I-like receptors; P2X7R, P2X purinoceptor 7; NF-κB, nuclear factor-kappa B; MAPK, mitogen-activated protein kinase; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3; PYD, pyrin domain; CARD, caspase activation and recruitment domain; ASC, apoptosis-associated speck-like protein containing CARD; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; IL, interleukin; GSDMD, gasdermin D.

upregulated within 12 hours of ischemic stroke, peaks at 24 hours, and remains high beyond 48 hours [35]. Neurons express NLRP3 in a constitutive manner but do not express IL-1 β and IL-18, indicating that NLRP3 can be assembled without its adaptor or effector proteins [36]. After ischemic stroke, NLRP3 is activated first in microglia and subsequently upregulated in endothelial cells and neurons [37]. NLRP3 regulates neuronal and glial cell death after ischemic stroke through caspase-1 and proinflammatory cytokines [28]. DAMPs derived from dying neurons and mitochondrial reactive oxygen species (ROS) are the major stimulants for upregulation and activation of NLRP3 in ischemic stroke [35]. Activation of NLRP3 triggers formation of mature IL-1 β and IL-18 by caspase-1 and matrix metalloproteinases signaling in a time-dependent manner following ischemic stroke. NLRP3 assembly and activation are based on two serial phases associated with cell injury (Figure 1). First, a priming phase is required for upregulation of transcription and translation of NLRP3-inflammasomal components and the pro-IL-1/pro-IL-18 through NF- κ B and MAPK, which are activated by various PRRs such as TLRs and RAGE and their downstream signaling proteins [38]. Then, an activation phase follows the priming phase and results in assembly of the NLRP3 inflammasome complex through multiple inducers including ATP, K⁺ efflux, ROS, mitochondrial DNA, calcium overload, and lysosome rupture [39,40]. ATP released through pannexin hemichannels in neurons or astrocytes activates P2X purinoceptor 7 (P2X7R), which mediates K⁺ efflux and amplifies the inflammasome pathway [41]. Mitochondrial ROS induce the ROS scavenging thioredoxin-interacting protein, which activates oligomerization of NLRP3 [42]. Notably, in recurrent stroke, primed NLRP3 can exacerbate ischemic injury together with preformed ASC [43]. Furthermore, NLRP3 has been suggested to promote atherosclerotic inflammation and thereby increase the risk of ischemic stroke [44].

Modulation of NLRP3 inflammasome activation at various levels could be the basis for possible therapeutic targets for ischemic stroke. Inhibition of NLRP3 inflammasome activation through genetic depletion [45] or pharmacological inhibition with a mitochondrial stabilizer [46], TPEN (a membrane-permeant zinc chelator) [47], or an anti-caspase-1 antagonist [33] have been reported to attenuate acute ischemic stroke injury by decreasing the inflammatory response, BBB leakage, edema, infarct volume, and functional deficit. However, reports on the role of NLRP3 in the pathogenesis of ischemic stroke are conflicting. Denes et al. [32] showed that NLRP3 was not involved in the acute phase of focal cerebral ischemia. Multiple inflammasome components such as NLRC4, AIM2, ASC, and CARD

mediate inflammatory responses independent from NLRP3 during ischemic brain injury. IL-1 β upregulation and peripheral inflammatory cell infiltration are also independent of NLRP3. Furthermore, the first event of ischemic stroke was associated with NLRC4 and AIM2 but not with NLRP3 [43]. More recently, specific inhibition of NLRP3 with MCC950 or depletion of NLRP3 did not reduce ischemic brain damage [48]. There are several explanations for this conflicting observation. Differences in stroke models used, occlusion duration, and intervention modality can modify the inflammatory response. In contrast to NLRP3, AIM2 and NLRC4 do not require two-step processing for activation [30,32] and can respond immediately to the first ischemic hit. Following recurrent stroke, NLRP3 might be in an activated state and ready to boost the inflammatory pathway [43].

NLRP1

NLRP1 was the first inflammasome, characterized 20 years ago. Oxygen and glucose deprivation increase NLRP1 expression in neurons through ATP depletion [49]. The NLRP1 inflammasome is expressed in neurons and astrocytes in a preassembled state [25]. Furthermore, NLRP1 expression is increased markedly in neurons and microglia following ischemic stroke [50]. Administration of an anti-NLRP1 antibody significantly reduces infarct volume, indicating that NLRP1 plays a role in the pathogenesis of ischemic stroke [7]. Although inflammasomes have some overlap in function, the expression patterns differ based on cell type, injury type, and time course of injury. The NLRP3 inflammasome is expressed mainly in microglia, which propagates the inflammatory response [51]. However, the NLRP1 inflammasome is expressed in neurons, indicating that it mediates neuronal pyroptosis [52]. Both NLRP1 and NLRP3 could be important targets to achieve effective neuroprotection via inhibition of neuronal pyroptosis and the inflammatory storm.

AIM2 and NLRC4

AIM2 and NLRC4 are regulated by various metabolic molecules and play a pathogenetic role after ischemic stroke. The AIM2 inflammasome consists of the PYD and DNA-binding HIN domains, which interact with double-stranded DNA [53]. AIM2 is involved in axonal and dendritic growth of neurons after ischemic stroke [54]. NLRC4 inflammasome is flanked by CARD, interacting with procaspase-1 [55]. NLRC4 is activated by bacterial molecular patterns, such as flagellin [56], and by DAMPs under hyperosmotic milieu, which might be associated with ischemic stroke [57]. AIM2 and NLRC4 are activated to mediate the inflammatory response as well as pyroptotic cell death in microglia during ischemic stroke [58]. To investigate the role of inflam-

masomes in the pathogenesis of ischemic stroke, genetic and pharmacological strategies have been used. Following ischemic stroke, inflammatory responses and infarct volumes were lower in AIM2, ASC, or NLRP3 knockout mice than in wild-type mice, indicating that AIM2 and NLRP3 contribute to brain damage after ischemic stroke [32].

Clinical evidence for involvement of inflammasomes in ischemic stroke

Based on clinical stroke research, multiple inflammasomes are involved in the pathogenesis of ischemic stroke. For example, NLRP3 is expressed in human brain tissues after stroke. In addition, caspase-1, IL-1 β , and IL-18 expression levels were upregulated in postmortem brain tissue of stroke patients [33,59]. In addition to brain tissue, human serum and serum-derived extracellular vesicles were investigated to track inflammasome component levels of caspase-1, ASC, IL-1 β , and IL-18 [60]. The research indicated that ASC in serum-derived extracellular vesicles is a potential biomarker in stroke, and the inflammasome participates in pathogenesis of brain ischemia in humans.

Inflammasome as a therapeutic target in ischemic stroke

Potential targets

The inflammasome is involved critically in a cascade of sterile inflammation and irreversible cell damage during cerebral ischemia. Therefore, inhibition of individual or multiple inflammasomes would be an effective method for treating ischemic stroke. The inflammasomes activate caspase-1, which transforms pro-IL-1 and pro-IL-18 into mature inflammatory cyto-

kines and interacts with other brain cells. This process is driven by increased expression of inflammasome components, assembly, activation, and secretion. Therefore, upstream or downstream molecules regulating the inflammasome pathway are promising targets as therapeutics to manage ischemic stroke (Table 1). Specifically, NF- κ B and MAPK signaling molecules; proteins of the inflammasome complex; and BTK, IL-1 β , IL-18, and caspase-1 serve as potential targets. Because mitochondrial ROS and DNA can trigger inflammasome activation, stabilization of mitochondrial permeability using nitric oxide might be an effective method to suppress inflammasome-mediated inflammation [61]. In addition, ATP, pannexin hemichannels, and P2X7Rs, which activate the inflammasome via K⁺ efflux, could be effective targets for modifying the inflammasome pathway [41].

Therapeutics under development

Several chemicals, including BAY-11-7082 (NF- κ B inhibitor), SB 203580 (p38-MAPK inhibitor), probenecid (pannexin-1 inhibitor), CY-09, MCC950, glyburide (NLRP3 inhibitor), VX765 (selective inhibitor of caspase-1), brilliant blue G (P2X7R antagonist), ibrutinib (selective BTK inhibitor), and nitric oxide (mitochondrial stabilizer), have been explored for inhibitory effects on the inflammasome pathway. Several of these compounds showed promising therapeutic efficacy in animal models of ischemic stroke and are under clinical translation [34,62-66]. In addition, microRNAs, including miR-22 and miR-132, could influence the NLRP3 pathway via epigenetic modification [67]. Drugs on the market have been used in other ways than initially designed to affect various levels of inflammasome pathways. For example, intravenous immunoglobulins reduced NLRP1,

Table 1 Strategies to modulate or inhibit inflammasomes

Target	Agent	Action and outcome in the model
NF- κ B	Bay-11-7082	Inhibit priming signal of inflammasome
NLRP3	MCC950, glyburide, CY-09, miR-22, miR-132	Inhibit NLRP3 oligomerization, but contradictory results in focal cerebral ischemia
Mitochondrial membrane	TPEN, nitric oxide	Inhibit NLRP3 oligomerization
ASC	ASC antibody	Inhibit expression of caspase-1 and XIAP
Caspase-1	VX-765, Ac-YVAD-cmk	Inhibit caspase-1, IL-1 β , and IL-18
NLRP1	NLRP1 antibody	Inhibit NLRP1 and downstream caspase-1 and IL-1 β
GSDMD	Disulfiram, LDC7559	Inhibit GSDMD-dependent cell lysis
Bruton's tyrosine kinase	Ibrutinib (PCI-32765)	Reduce IL-1 β , IL-6, and microglia activation
P2X7	Brilliant blue G	Inhibit K ⁺ efflux and inflammasome activity
Pannexin channel	Probenecid	Inhibit ATP binding, K ⁺ efflux, and inflammasome activity
GPCR19	INT-777	Activate GPCR19 and attenuate neuronal degeneration and oligodendrocyte death

NF- κ B, nuclear factor-kappa B; NLRP3, nucleotide-binding oligomerization domain-like receptor (NLR) pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing caspase activation and recruitment domain; XIAP, X-linked inhibitor of apoptosis protein; IL, interleukin; NLRP1, NLR pyrin domain containing 1; GSDMD, gasdermin D; P2X7, P2X purinoceptor; ATP, adenosine triphosphate; GPCR19, G protein-coupled receptor 19.

NLRP3, ASC, IL-1, and IL-18 expression, thereby improving neurological outcomes of ischemic stroke [33]. The steroids 17 β -estradiol and progesterone decreased the NLRP3, ASC, and NLR4 levels and reduced infarct volume [68]. Statin and melatonin have conferred an immunomodulatory effect in stroke through inhibition of the NLRP3 inflammasome [69]. Edaravone, a free radical scavenger, suppressed NF- κ B-dependent NLRP3 activation and alleviated acute brain injury [70]. Natural products, including resveratrol, paeoniflorin, curcumin, and sinomenine, also improved neurological outcomes following ischemic stroke by inhibiting the inflammasome pathway at multiple levels [71].

GPCR19/TGR5 agonist

The G protein-coupled receptor 19 (GPCR19) is a membrane-type receptor for bile acids and is termed G protein-coupled bile acid receptor 1 or Takeda G protein-coupled receptor 5 [72]. GPCR19 is implicated in the regulation of energy homeostasis and immune suppression by elevating intracellular cyclic adenosine monophosphate (cAMP) and subsequently increasing anti-inflammatory growth factors and neuropeptides. Thus, GPCR19 is regarded as a potential therapeutic target for metabolic disorders, ischemia-reperfusion injury, and inflammatory diseases such as experimental autoimmune encephalomyelitis [73]. Tauroursodeoxycholic acid (TUDCA), a bile acid conjugate, binds to GPCR19 and mediates neuroprotection from acute brain injury via anti-inflammatory effects [74]. The protective effects of GPCR19 on BBB integrity have been demonstrated in ischemic stroke. GPCR19 is expressed in neurons, astrocytes, and microglia [75], and the GPCR19 level is increased in the ischemic brain, especially in microglia. TUDCA suppressed microglial activation, induced TGF- β signaling, and inhibited NF- κ B activation [76,77]. INT777, a GPCR19 agonist, protected BBB disruption and improved functional outcome after ischemic stroke, whereas siRNA silencing of GPCR19 worsened BBB leakage and outcome [78].

A GPCR19 agonist is involved in the two-step pathway for NLRP3 inflammasome activation; this agonist blocks both NF- κ B-dependent priming phase and ATP/P2X7R/K⁺ efflux-dependent NLRP3 activation phase [79]. An increased intracellular cAMP level elicits ubiquitination of NLRP3, inhibiting NLRP3-ASC inflammasome complex formation. Furthermore, increase in cAMP suppresses bone marrow-derived myeloid cell recruitment and blood cell infiltration into the brain [80]. Consequently, inhibition of the inflammasome pathway through activation of GPCR19 would be fast and robust, as well as selective and safe. Thus, a GPCR19 agonist would be more advantageous

than other inflammasome modifiers in ischemic stroke, especially because ischemic stroke is complicated by heterogeneous mechanisms and patient comorbidities. As mentioned above, because data regarding the efficacy of inflammasome inhibitors in ischemic stroke are conflicting, an inflammasome modulator with the most upstream target, GPCR19, is needed.

Future directions

The understanding of ischemic stroke mechanisms has significantly increased in recent years. The ideal therapeutic strategy for ischemic stroke is reperfusion with intravenous thrombolysis or endovascular thrombectomy followed by treatment with an effective neuroprotectant such as antiexcitotoxic agent, free radical scavenger, anti-inflammatory agent, or a combination of these. Sterile inflammation contributes significantly to ischemic brain damage. Various types of inflammasomes are activated in different cell types by early molecular inducers, such as DAMPs and ROS, and act as central effectors of sterile inflammation, leading to cell death through pyroptosis. Increasing evidence has shown that blocking or inhibiting inflammasome signaling pathways can rescue the ischemic brain. However, the efficacy data were somewhat conflicting, and the benefits over other anti-inflammatory approaches remain unclear. Future studies should focus on more effective targets in the inflammasome signaling pathway and optimal timing of drug administration. In addition, it is necessary to identify the physiological function of inflammasomes to balance the efficacy and potential adverse effects. Furthermore, the effectiveness of inflammasome targeting might be influenced by patient characteristics such as age, severity, stroke type, reperfusion status, tPA use, comorbidities, or previous stroke. Therefore, the patient population that would benefit most from inflammasome-targeted treatments should be determined.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

Conceptualization: Jung KH, Seong SY; Funding acquisition: Jung KH; Writing-original draft: Jung KH, Seong SY; Writing-review & editing: Jung KH.

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