Evidence and perspective for the role of the NLRP3 inflammasome signaling pathway in ischemic stroke and its therapeutic potential (Review)

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Abstract. Ischemic stroke is one of the main causes of death and disablement globally. The NLR family pyrin domain containing 3 (NLRP3) inflammasome is established as a sensor of detecting cellular damage and modulating inflammatory responses to injury during the progress of ischemic stroke. Inhibiting or blocking the NLRP3 inflammasome at different stages, including expression, assembly, and secretion, may have great promise to improve the neurological deficits during ischemic stroke. The current review provides a comprehensive summary of the current understanding in the literature of the molecular structure, expression, and assembly of the NLRP3 inflammasome, and highlights its potential as a novel therapeutic target for ischemic stroke.

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1. Introduction

Stroke is the third-leading cause of death, following coronary heart disease and cancer, and the main cause of permanent adult disablement in most Western countries (1). In China, it has become the first leading cause of death in recent years (2). Generally, between two main types of stroke, ischemic and hemorrhagic, ischemic stroke accounts for the majority of cases (~80-90%) (3-5). Ischemic injury often leads to irreversible cerebral infarction depending on the location, severity, and duration of cerebral blood flow (CBF) reduction, causing cognitive and motor impairment. Based on the pathophysiological characteristics of ischemic stroke, there are currently two major therapeutic strategies: Reperfusion and neuroprotection. Using thrombolytic, antithrombotic and anti-aggregation agents, the blood flow of the compromised region can be restored. However, only one drug, IV Alteplase, is recommended to treat ischemic stroke, and the drug has a very short therapeutic time window and a high risk of hemorrhagic transformation, which severely limits its clinical use (6). The second therapeutic strategy, neuroprotection, aims at preventing neuronal death by regulating multiple intra- or extracellular signals that lead to cell injury. The concept that ischemic penumbra is potentially salvageable is the basis of numerous searches for neuroprotective medications that can save the penumbra tissue and limit the negative consequences

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of stroke (7). However, no drug with proven neuroprotective efficiency and without harmful adverse side effects has been discovered yet. Therefore, it is urgent to develop novel drugs with potential and effective targets.

Inflammation is a defense response aiming at eliminating the primary causes of cell damage. Although inflammation aids in clearing infections and other toxic stimuli, inflammatory responses in the cerebral tissue during an ischemic injury increase the damage for a few hours following the onset of cerebral ischemia (8). During transient or permanent vascular obstruction, all the functions and molecules in the neurons, glial cells (oligodendrocytes, microglia and astrocytes) and vascular cells (endothelial cells and pericytes), identified as components of the neurovascular unit, are affected (9). Dangerous molecular signals are released from the damaged cells following multiple types of cellular stress (10). These signals, including danger signals denominated damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), activate the innate immune system via intracellular and extracellular pattern recognition receptors (PRRs), which have been highlighted recently as an important inflammatory mechanism that may contribute to cerebral ischemic injury (11). Inflammasomes are intracellular protein complexes associated with innate immunity. Activated inflammasomes are able to cleave the precursor of interleukin (IL)-1β and IL-18 to mature forms and initiate a newly discovered programmed inflammatory cell death, pyroptosis, via cleaved caspase-1 (12). In specific, the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family pyrin domain containing 3 (NLRP3), which is plentifully expressed in the brain, is regarded as one of the predominant inflammasomes, due to its critical role in recognizing cellular damage and modulating inflammatory responses to ischemia reperfusion injury during ischemic stroke (13). In the current review, we survey the existing evidence for the structure of NLRP3 inflammasome, its activation process in the ischemic brain and the therapeutic potential of blocking or inhibiting NLRP3 inflammasome signaling.

2. Overview of NLRP3 inflammasome complex

The inflammasome was first proposed in 2002 as an activator complex of caspase-1 that generates IL-1β via cleavage of its preform (14). Inflammation is now considered as an important innate immune reaction to multiple types of infection and tissue injury (15). Two kinds of inflammasomes have been established to date: Pyrin and HIN domain-containing protein (PYHIN) inflammasomes and NLR inflammasomes (12). NLRP3 inflammasome is one of the best characterized inflammasomes to date and the most relevant to aseptic inflammation (16). NLRP3, also known as NALP3, is encoded by the cold-induced auto-inflammatory syndrome-1 (*CIAS-1*) gene and is plentifully expressed in cells of the nervous and immune systems (17). It is commonly comprised of three domains: A C-terminal domain containing leucine-rich repeats (LRRs), a central NACHT domain, and an N-terminal PYD. The LRR domain is implicated in the progress of ligand sensing (18,19). The NACHT domain is responsible for oligomerization and assembly of the central core of the NLRP3 inflammasome, which depends on ATP (20). The

N-terminal PYD domain encourages homotypic PYD/PYD interactions with apoptosis-associated speck-like protein containing CARD (ASC) (21,22), which comprises both PYD and CARD domains and regulates inflammatory response and cell death (23,24). Following danger signals, activation of upstream signals and oligomerization of NLRP3 result in the formation of NLRP3 inflammasome. The NLRP3 inflammasome consists of three cytoplasmic proteins: NLRP3, ASC and the precursor of caspase-1 (25,26). Once binding to NLRP3 via PYD domain, ASC recruits the precursor of caspase-1 via homotypic CARD/CARD interactions (22). Subsequently, activated caspase-1 is generated, which then cleaves the precursor of proinflammatory cytokines IL-1β and IL-18, and results in the maturation and secretion of these cytokines, inducing cellular pyroptosis (Fig. 1) (27). Recently, multiple studies have reported that excessive activation of NLRP3 inflammasome is closely associated with pathophysiological changes in a majority of inflammatory and non-inflammatory illnesses, including ischemic stroke (28-31).

3. Activation and role of NLRP3 inflammasome in ischemic stroke

An increasing number of studies have indicated a key role of NLRP3 inflammasome in recognizing cellular damage and modulating inflammatory responses that eventually result in cell death (19,32). The NLRP3 inflammasome was first associated with ischemic injury in an animal model of renal ischemic injury, which occurs as blockage of blood flow (33,34). Following renal ischemic injury, plenty of evidence proves that activation of NLRP3 is critical in mediating myocardial and liver ischemic damage (35). In the central nervous system (CNS), NLRP3 inflammasome was first reported to be activated in cortical neurons under ischemic conditions and the expression of NLRP3, ASC, caspase-1, IL-1β and IL-18 was upregulated *in vitro* and *in vivo* (36). The latter study also demonstrated that suppression of NLRP3 inflammasome activity and neuroprotection resulted from intravenous immunoglobulin (IVIg) and anti-caspase-1 treatment, respectively (36). Another study indicated that deficiency of the NLRP3 gene protected mice from ischemic damage with improved functional outcomes, decreased infarction volume and edema formation, preserved blood brain barrier (BBB) permeability, and reduced inflammatory pathology in a transient middle cerebral artery occlusion (tMCAO) mouse model (37,38), which was first developed in 1986 to mimic ischemic stroke in patients by Koizumi (39). However, several researchers have questioned the role of NLRP3 in the progress of cerebral ischemic injury (40). The conflict between these results could be attributed to differences in ischemic time that may modify the inflammatory response.

After the NLRP3 inflammasome is activated, caspase-1, an evolutionarily conserved enzyme that proteolytically cleaves other proteins, becomes mature. Following ischemic injury in a permanent animal model of stroke, the levels of activated caspase-1 increase at 30 min following surgery, and a second wave of activation comes 12 h later (41). The upregulated levels of cleaved caspase-1 have been observed in neurons and astrocytes following cerebral ischemia, and in microglia 24 h after a stroke (42). Using transgenic mice, the role of caspase-1 has

been highlighted in the progress of ischemic stroke. Knockout or dominant-negative mutants of caspase-1 inhibited brain damage in contrast to wild type, following experimental stroke (43,44). The therapeutic effects of caspase-1 molecule inhibitors have also been observed in an oxygen/glucose deprivation model in rat hippocampal slices (45). Collectively, these studies demonstrate a critical role of caspase-1 during ischemic stroke.

Several studies have demonstrated upregulated concentrations of pro-inflammatory factors in the blood, cerebrospinal fluid, and location of blockages of the brain in both clinical patients and experimental animals (46-49), suggesting a localized CNS inflammatory response to ischemic injury. In pathological conditions, premature IL-1β and IL-18 proteins without biological function need to be processed and secreted to exert their pro-inflammatory effects (50). Secreted IL-1 in extracellular space has a direct impact on nearby neurons via IL-1 receptors. High concentrations of IL-1β stimulates phosphorylation and activation of the N-methyl-D-aspartate (NMDA) receptor, which induces excessive calcium flux and excitatory toxicity (51).

4. Expression of the NLRP3 inflammasome complex in the ischemic brain

The NLRP3 inflammasome complex, except for immune organs, has also been recently found to be expressed in the brain and spinal cord (47,50). Based on expression data from 11 types of tissues, it was demonstrated that the brain does not express IL-1β and IL-18 constitutively, but expresses NLRP3 inflammasome in a constitutive state, indicating that the NLRP3 inflammasome can be assembled without upregulation of one or two components (52). Expression of the member proteins of the NLRP3 inflammasome complex and IL-1β and IL-18 has been demonstrated to be upregulated in post-mortal brain tissue from stroke patients. A higher level of activated caspase-1 in ischemic brain tissues compared with control brain tissues from patients was further confirmed by immunohistochemical analysis (36,53). At the cellular level, similar to bone marrow-derived macrophages, microglia is the main cell type that expresses NLRP3, ASC and caspase-1 in the brain. However, neither NLRP3 nor ASC can be detected in astrocytes, which highlights the important role of microglia-dependent NLRP3 inflammasome activation under neuroinflammatory conditions (54). In 2014, for the first time, animals subjected to MCAO and cell cultures subjected to oxygen-glucose deprivation (OGD) modeling ischemia/reperfusion injury *in vivo* and *in vitro* were used to confirm NLRP3 expression in ischemic stroke. Another study has demonstrated that NLRP3-related proteins are expressed in endotheliocytes and microglia instead of neurons, suggesting that they are the main sources of NLRP3 in the location of the ischemic incident (38). Others found that the levels of NLRP3 inflammasome proteins were also upregulated in primary cortical neurons under ischemic injury (36). Although the specific expression pattern of NLRP3-related proteins in different types of cells in ischemic brain remains unclear, it is certain that NLRP3 inflammasome signaling has an important role in the pathogenesis of ischemic stroke, at the neurovascular unit level. The underlying causes for the differences in the distribution may due to diverse models, ischemic duration and different interventions.

5. Activation of the NLRP3 inflammasome pathway in ischemic stroke

The specific intracellular and extracellular signals leading to NLRP3 inflammasome activation are not yet fully understood. Evidence has revealed that assembly of NLRP3 inflammasome and activation of downstream signals depend on two complementary signals associated with cell injury: A priming signal, required for upregulated expression of the NLRP3 inflammasome complex proteins and the precursors of IL-1β and IL-18 through nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) signaling pathways (55); and a second signal that results in NLRP3 activation and ASC phosphorylation, thus triggering their assembly into the NLRP3 inflammasome complex (Fig. 2). In addition, multiple activation mechanisms have been described for the inflammasome, including K^+ efflux, reactive oxygen species (ROS) overproduction, mitochondrial dysfunction, Ca^{2+} overload, and lysosome rupture. Notably, these mechanisms also overlap with the two-step activation of the NLRP3 inflammasome. It is well-established that these mechanisms exist in the progress of ischemic stroke. Several experts have confirmed that reducing the ATP/ADP ratio following ischemic stroke opens the channel and allows K^+ ions to exit the cells via potassium channels (56). Mitochondrial dysfunction (57), ROS overproduction (58) and Ca^{2+} overload (59) are also known to have important roles in the amplification and transmission of ischemic injury.

NLRP3, pro-IL-1β and pro-IL-18 do not abound in physiological conditions, and therefore a priming signal, specifically the upregulation of NLRP3-related proteins, is necessary for the activation of NLRP3 inflammasome. In addition, several plasma membrane pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) and receptor for advanced glycation end-products (RAGE), and downstream adaptor proteins, such as myeloid differentiation primary response gene 88 (MyD88) and tumor necrosis factor receptor-associated factor 6 (TRAF6), may serve a role in activation of two phosphorylation signaling pathways, NF-κB and MAPK (60-63). Both of the latter signaling pathways are considered to regulate both the expression of the member proteins of the NLRP3 inflammasome complex, and the expression of the precursors of IL-1β and IL-18 during the inflammatory response to specific cellular stress (64-66).

As aforementioned, after the activating priming signal, ASC, an adaptor protein, recruits NLRP3 proteins and forms the NLRP3 inflammasome complex. Of all the intracellular and extracellular factors, the best-studied stimulus, K^+ efflux, has been demonstrated to have a role in activation of the NLRP3 inflammasome (67). This non-selective K^+ cation channel on the cell surface is able to change intracellular ionic contents depending on ATP binding and to activate downstream signals, inducing maturation and secretion of IL-1β (68). Previous studies have demonstrated that downregulated levels of intracellular K^+ are indispensable for activation of the NLRP3 inflammasome pathway when compared with other known stimuli, which highlighted the role of K^+ efflux in this process. P2X purinoceptor 7 (P2X7R) is one of the
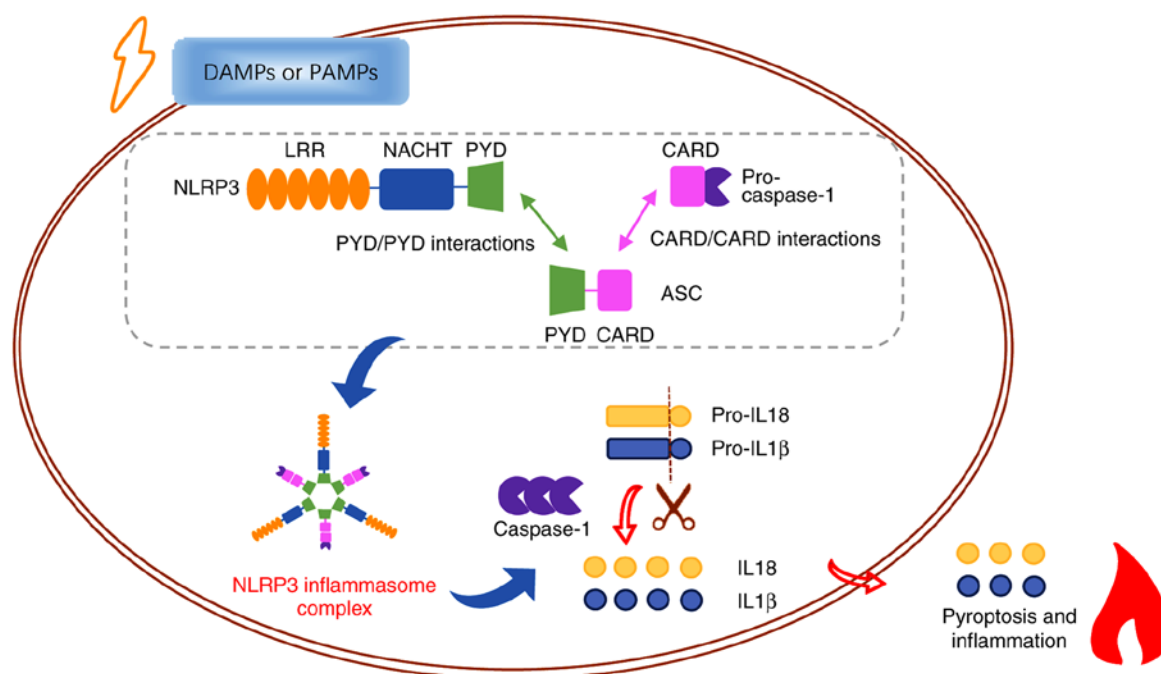
Figure 1. Structure of the NLRP3 inflammasome complex. The NLRP3 inflammasome complex consists of NLRP3, ASC and pro-caspase1. NLRP3 is composed of three domains: A C-terminal domain containing LRRs, a central NACHT domain, and an N-terminal PYD. The ASC protein is composed of a PYD and a CARD. Following DAMPs or PAMPs activating upstream signaling pathways, homotypic interactions between homotypic domains, such as NATCH/NATCH, PYD/PYD and CARD/CARD, have a key role in assembly of the NLRP3 inflammasome. Finally, pro-caspase1 is cleaved to form the bioactive caspase1, which then induces the maturation and secretion of IL-1β and IL-18, and subsequently pyroptosis. NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing CARD; LRRs, leucine-rich repeats; PYD, pyrin domain; CARD, caspase recruitment domain; DAMPs, danger associated molecular patterns; PAMPs, pathogen-associated molecular patterns; IL, interleukin.
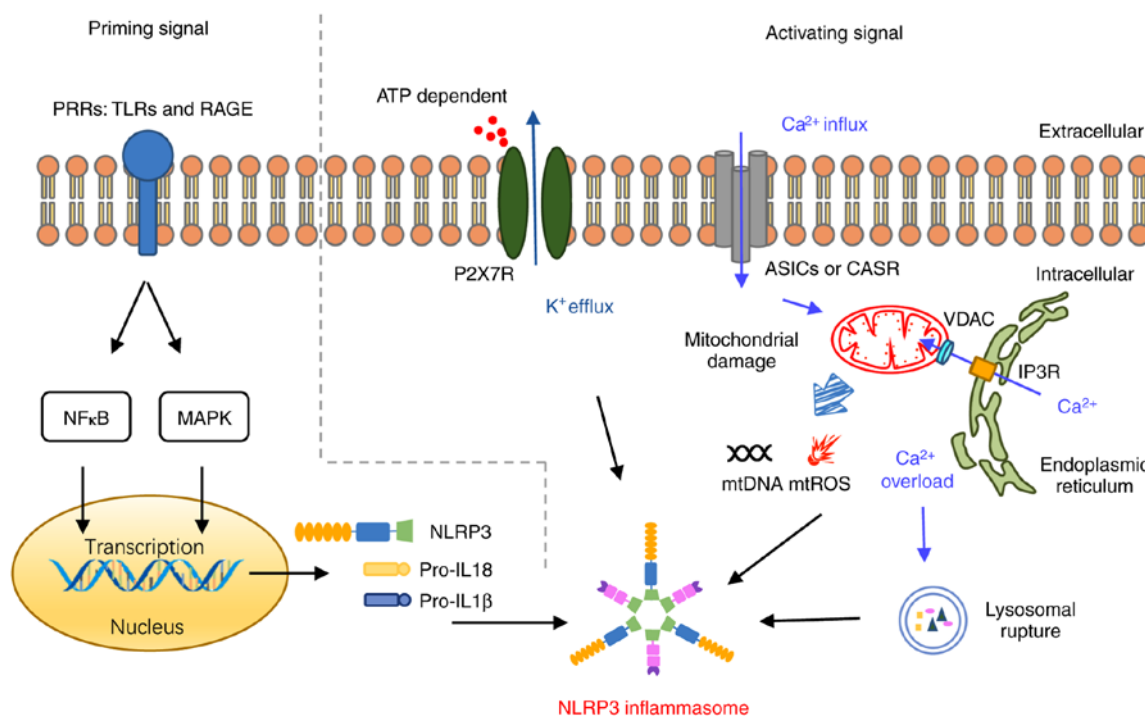

Figure 2. Activation of the NLRP3 inflammasome signaling pathway in ischemic stroke. Two steps of activation have been recognized: The priming and the activating. For the priming step, PRRs, such as TLRs and RAGE, are major initiative signals that upregulate the expression of the NLRP3 inflammasome complex proteins and of pro-inflammatory cytokines, via transcription factors such as NF-κB and MAPK. Following the priming step, ASC, an adaptor protein, recruits NLRP3 and forms the NLRP3 inflammasome complex. Finally, intracellular and extracellular stimuli, including disruption of K$^+$ and Ca^{2+} homeostasis, and mtDNA and mtROS release from mitochondrial dysfunction and lysosomal rupture, have been demonstrated to have important roles in activation of the NLRP3 inflammasome. NLRP3, NLR family pyrin domain containing 3; PRRs, plasma membrane pattern recognition receptors; TLRs, toll-like receptors; RAGE, receptor for advanced glycation end-products; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; ASC, apoptosis-associated speck-like protein containing CARD; mt, mitochondrial; ROS, reactive oxygen species; IL, interleukin; P2X7R, P2X purinoceptor 7; ASICs, acid-sensing ion channels; CASR, calcium-sensing receptor; VDAC, voltage-dependent anion channels; IP3R, inositol 1,4,5-trisphosphate receptor.

best-characterized receptors associated with K^+ efflux, whose activation might induce release of pro-inflammatory cytokines and amplify ischemic injury via ATP. Experimental evidence from P2X7R knockdown mice confirmed the role of this receptor in NLRP3 inflammasome response (69).

Mitochondrial damage is another important activation mechanism of the NLRP3 inflammasome. The mitochondrion is a double-membrane-bound intracellular organelle and the predominant location in the cell that produces both energy and reactive oxygen species (ROS) (70). Previous research has indicated that high levels of ROS under multiple kinds of cellular stress, particularly those produced by mitochondria (mtROS), activates the NLRP3 inflammasome signal pathway (71-74). In detail, high levels of ROS induce the ROS scavenging protein thioredoxin resolving from thioredoxin interacting/inhibiting protein (TXNIP), which then directly binds with NLRP3 proteins and modulates its assembly via oligomerization (75-77). Although several investigations have demonstrated that TXNIP is necessary for NLRP3 inflammasome assembly, a cell type-specific modulation of TXNIP occurs, which limits its mediation of inflammatory response ROS signaling pathway activation to particular cell types (78-80). In addition to mtROS, dysfunctional mitochondria also release mitochondrial DNA (mtDNA) into the cytoplasm, directly inducing the assembly of NLRP3 inflammasome complex via molecular self-association (81-83). Of note, appropriate amount of nitric oxide, another kind of free radical, is able to trigger a cascade to modulate mitochondrial permeability, limit mtROS releases and suppress NLRP3 inflammasome activation (84,85).

Previous evidence has demonstrated that intracellular level of Ca^{2+} is associated with the activation of NLRP3 inflammasome, which highlights the role of calcium homeostasis in NLRP3 inflammasome signaling. An early study reported that increased levels of intracellular Ca^{2+} along with decreased levels of K^+ activate the NLRP3 inflammasome pathway and release mature IL-1β and IL-18 via P2X7R, as aforementioned (86). Later, it was demonstrated that both suppression of extracellular Ca^{2+} influx via membrane receptor and inhibition of intracellular Ca^{2+} overload depending on endoplasmic reticulum (ER) were able to block NLRP3 inflammasome activation and IL-1β maturation and secretion by ATP, nigericin, and alum (87), indicating a key role of Ca^{2+} level in NLRP3 inflammasome activation (88). Notably, via Ca^{2+} overload, mitochondrial damage and ER stress are among the best-described pathological factors activating the NLRP3 inflammasome pathway. Extracellular Ca^{2+} may enter the cell trough plasma membrane-resident Ca^{2+} channels called acid-sensing ion channels (ASICs) (89,90). The calcium-sensing receptor (CASR) has also been reported to increase the concentration of intracellular Ca^{2+}, initiate the phospholipase C pathway via the intracellular side and induce assembly of the NLRP3 inflammasome complex (91). Mitochondria-associated membranes (MAM), a mechanism resulting from crosstalk between ER and mitochondria, are involved in Ca^{2+} flux from ER to mitochondria via inositol 1,4,5-trisphosphate receptor (IP3R) and mitochondrial voltage-dependent anion channels (VDAC) (92,93). Pathologically high levels of Ca^{2+} can induce NLRP3 inflammasome activation via two different mechanisms: By enhancing mtROS production,

with associated mitochondrial destabilization (94); and by inducing lysosomal rupture, resulting in lysosome contents being released into the cytosol, particularly cathepsin B, which binds to the LRR domain of NLRP3 and triggers NLRP3 signaling (95,96). Cathepsin B is also activated by the TAK1 kinase and is involved in the activation of MAPKs, one of which participates in the priming step to activate the NLRP3 inflammasome (97-99).

6. Inhibitors targeting the NLRP3 inflammasome pathway for ischemic stroke treatment

Accumulating evidence indicates that the NLRP3 inflammasome has an important role in ischemic brain injury. Targeting upstream or downstream of the NLRP3 inflammasome pathway at the molecular level, including modulating protein expression, assembly, activation and/or secretion, may have a promising prospect in developing novel therapeutic agents for ischemic stroke (Table I). Core proteins involved in the NF-κB and MAPK pathways, proteins of the NLRP3 inflammasome complex, plasma membrane receptors or channels, cytokines (IL-1β and IL-18) and their receptors may serve as potential therapeutic targets for ischemic stroke (19). A previous study focused on the effect of Bay-11-7082 (an NF-κB inhibitor), SB 203580 (a P38-MAPK inhibitor), JNK Inhibitor V (a JNK inhibitor), and U-0126 (an ERK inhibitor) on a mice tMCAO model and in neurons undergoing OGD, and demonstrated that all of these inhibitors protected neurons during simulated ischemia, via attenuating the levels of NLRP3 inflammasome complex, and via inhibiting activation of NLRP3 inflammasome and maturation of IL-1β and IL-18 (100). Probenecid, a pannexin 1 inhibitor, has also been found to induce astrocyte death and ROS generation, attenuate expression levels of NLRP3 and inhibit the extracellular release of IL-1β (101). MCC950 and glyburide, both NLRP3 oligomerization inhibitors, reduce infarction volume, neuronal apoptosis, and neurological impairment, and have anti-oxidative stress and anti-inflammatory effects, respectively (102-105). Recently, several natural compounds, including resveratrol, paeoniflorin and sinomenine, were also demonstrated to reduce cerebral infarction volume, decrease brain water content, improve neurological scores and grip strength, and prevent neuronal cell death following ischemic stroke, through downregulating the expression of the components of the NLRP3 inflammasome and their downstream proteins and attenuating its activation (106-108). However, there is little clinical data on the effects of these agents to date. Further studies are required to examine the efficiency and safety of these agents in the clinic in the future.

Although targeting the NLRP3 inflammasome pathway rather than anti-ischemic systems may develop promising therapeutic strategies to treat ischemic stroke, several aspects of this potential treatment must be clarified. Firstly, the specific expression pattern of NLRP3 activation in specific cell types and brain regions following ischemic injury require further investigation. Additionally, whether the upstream pathways are different among various cell types remains to be elucidated. Secondly, it is well known that whether inflammation will have destructive or beneficial effects depends on the severity, frequency, and duration of ischemic injury. It is

Table I. Preclinical evidence of drugs targeting the NLRP3 inflammasome to treat ischemic stroke.

Drug name	Characteristic	Therapeutic effects	Stroke model	(Refs.)
Apocynin	NOX inhibitor	1. Reduced infarction volume, improved post-stroke survival, and recovery of neurological functions 2. Reduced levels of NOX2, NOX4, and ROS 3. Inhibition of IκBα degradation, nuclear localization of NF-κBp65, and expression of its target genes (COX2 and iNOS) 4. Suppressed expression of NLRP3, ASC, caspase-1, IL-1β, and IL-18	Mouse tMCAO model	(120)
Probenecid	Pannexin 1 inhibitor	1. Induced astrocyte death and ROS generation 2. Attenuated expression levels of NLRP3 and caspase-1 3. Inhibited extracellular release of IL-1β	Primary astrocyte OGD	(101)
Minocycline	Antibiotic immunosuppressor	1. Improved neurological function, reduced infarction volume, and alleviated cerebral edema 2. Prevented activation of microglia and attenuation of NLRP3 inflammasome signaling following tMCAO injury 3. Inhibited signals 1 and 2 of NLRP3 inflammasome activation in BV2 cells.	Mouse tMCAO model and BV2 cell OGD	(111)
Bay-11-7082, SB 203580, JNK Inhibitor V, U-0126	NF-κB inhibitor, P38-MAPK inhibitor, JNK inhibitor, ERK inhibitor, respectively	1. Protection of neurons during simulated ischemia 2. Attenuated expression of inflammasome proteins, IL-1β and IL-18 3. Attenuated inflammasome activation	Mouse tMCAO model and primary neuron OGD	(100)
Nicorandil	K$^+$ ATP channel opener	1. Increased tumor necrosis factor-α and IL-1β levels 2. Inhibited activation of NLRP3 inflammasome and TLR4 signaling pathways	BV2 cell OGD	(121)
Nafamostat mesilate	Synthetic serine protease inhibitor	1. Reduced brain infarction volume and improved sensorimotor and cognitive function 2. Altered expression profiles of inflammation mediators and induced expression of anti-inflammatory mediators 3. Inhibited thrombin-stimulated NLRP3 inflammasome activation 4. Decreased invasion of inflammatory cells	Rat tMCAO model and primary microglial culture OGD	(122)
MCC950	NLRP3 oligomerization inhibitor	1. Reduced infarction volume, neuronal apoptosis, and neurological impairment 2. Inhibited neuronal apoptosis 3. Inhibited platelet activation/aggregation and thrombus formation *in vitro*	Photothrombotic ischemia mice, primary neuron OGD, and platelet aggregation	(102,103,105)

Table I. Continued.

Drug name	Characteristic	Therapeutic effects	Stroke model	(Refs.)
Glyburide	NLRP3 oligomerization inhibitor	Anti-oxidative stress and anti-inflammatory effects	PC12 cell OGD	(104)
Sinomenine	Natural alkaloid compound	1. Alleviated cerebral infarction, brain edema, neuronal apoptosis, and neurological deficiency 2. Attenuated astrocytic and microglial activation in the ischemic hemisphere 3. Inhibited NLRP3, ASC, cleaved caspase-1, and pro-inflammatory cytokines 4. Activated the AMPK pathway	Mouse tMCAO model and primary mixed glial cell OGD	(108)
Ruscogenin	Natural steroid sapogenin	1. Decreased brain infarction and edema, improved neurological deficit, increased cerebral brain flow, ameliorated histopathological damage, reduced Evans blue leakage, and upregulated expression of TJs 2. Increased cell viability and trans-endothelial electrical resistance value, decreased sodium fluorescein leakage, and modulated TJ expression 3. Inhibited expression of IL-1β, caspase-1 and NLRP3 4. Decreased ROS generation and MAPK activation	Mouse tMCAO model and bEnd.3 cells OGD	(123)
Umbelliferone	Natural coumarin derivative	1. Inhibited oxidative stress and production of inflammatory cytokines in brain tissues 2. Suppressed NLRP3 inflammasome activation via reduced expression of TXNIP.	Rat tMCAO model	(124)
Curcumin	Natural polyphenolic compound	1. Reduced brain infarction volume and attenuated neuronal damage 2. Inhibited IRE1α and PERK phosphorylation, suppression of intracellular ROS production, and increased AMPK activity. Knockdown of AMPKα with specific siRNAs abrogated its inhibitory effects on IRE1α and PERK phosphorylation 3. Reduced TXNIP expression and inhibited NLRP3 inflammasome activation by downregulation of NLRP3 and cleaved caspase-1 induction, and thus reduced IL-1β secretion	Rat tMCAO model, hippocampus OGD, and hippocampus glutamate stimulation	(125)
Chrysophanol	Natural anthraquinone derivative	1. Reduced neurological deficit, infarct size, brain edema and BBB permeability 2. Inhibited caspase 1/3, ASC, IL-1β, and NLRP3 expression	Mouse tMCAO model	(126)

Table I. Continued.

Drug name	Characteristic	Stroke model	Therapeutic effects	(Refs.)
17β-Estradiol	Natural steroid hormone	GCI mice and rat tMCAO model	1. Reduced levels of inflammasome proteins NLRC4, AIM2, ASC, and NLRP3 and of IL-1β, IL-18, and TNFα transcripts 2. Regulation of NLRP3 inflammasome activation and its downstream products, cleaved caspase-1 and IL-1β	(127,128)
Arctigenin	Natural lignan compound	Rat MCAO model and primary neuron OGD	1. Decreased infarction volume, neurological score, and brain water content 2. Inhibited cerebral ischemia and induced NLRP3 inflammasome activation	(129)
Sulforaphane	Natural organosulfur compound	Rat MCAO model	1. Reduced infarction volume and improved neurological scores 2. Reduced neutrophil infiltration 3. Inhibited NLRP3 inflammasome activation, downregulation of cleaved caspase-1, reduced IL-1β and IL-18 expression	(130)
Astragaloside IV	Natural triterpenoid saponin compound	Bilateral common carotid artery occlusion in mice	1. Reduced expression of TLR4 and its downstream adaptor proteins, including MyD88, toll/interleukin-1 receptor-domain containing adaptor-inducing interferon-β (TRIF), and tumor necrosis factor receptor associated factor-6 (TRAF6), and subsequently inhibited NF-κB phosphorylation. 2. Suppressed NLRP3 inflammasome activation by controlling ROS production 3. Reduced overactivation of microglia and overexpression of inflammatory cytokines in the hippocampus 4. Improved memory	(131)
Resveratrol	Natural polyphenolic compound	Mouse endothelin-1-induced MCAO model	1. Inhibited TXNIP expression and protection of the brain against ischemic damage. 2. Inhibited TXNIP expression and protection of the brain against ischemic damage. 3. Attenuated activation of PARP, NLRP3, caspase 1/3 and release of IL-1β 4. Reduced cerebral infarction volume, decreased brain water content, improved neurological scores and grip strength	(106,132)
Paeoniflorin	Natural bioactive monoterpene glucoside	Hippocampal slices OGD	1. Prevented neuronal cell death 2. Downregulated levels of components of NLRP1 and NLRP3 inflammasomes (NLRP1, NLRP3, ASC, and caspase-1) as well as their downstream proteins (IL-18, IL-1β and caspase-3)	(107)

possible that early immune responses may aggravate ischemic injury, while late responses may help to repair the damaged region. Therefore, early inhibition of the NLRP3 inflammasome pathway may be a beneficial and reasonable choice. On the other hand, using animal models, some anti-neuroinflammation agents have been demonstrated to protect ipsilateral brain against ischemic injury up to 7 days following ischemic induction (109-111), which highlights the long-term effect and application prospect of immunotherapy. Of note, intermittent fasting has been reported to attenuate NLRP3 pathway activity and repair damaged tissues up to 4 months following ischemic stroke (112). Future studies should focus on the optimal timing of NLRP3 inhibitor administration and explain the mechanisms behind damaging and protective actions of the immune system in ischemic stroke.

Finally, after glyburide was first found to selectively inhibit activation of the NLRP3 inflammasome without interfering with other inflammasome pathways (113), two other selective and direct NLRP3 inhibitors, CY-09 (114) and CP-456,773 (115,116), were recently identified. These drugs might provide a novel avenue toward therapeutic inhibition of the NLRP3 inflammasome, although their effect in ischemic stroke remains to be determined. Many NLRP3 inhibitors found to have benefits following ischemic stroke were identified as natural compounds, and further research should elucidate the relationship between molecular structure and anti-NLRP3 inflammasome function. Furthermore, several microRNAs have been demonstrated to influence the NLRP3 inflammasome pathway, including miR-22 (117), miR-132 (118), and long non-coding RNA XLOC_000647 (119). Further studies should consider the modulation of the NLRP3 inflammasome post-stroke via epigenetic approaches.

7. Conclusions

In conclusion, illuminating the role of NLRP3 inflammasome signaling pathways in ischemic injury will provide significant knowledge and opportunities to clarify the relationship between the innate immune system and the nervous system in the pathophysiology of ischemic stroke. Above all, more and larger clinical studies examining the associations between clinical symptoms and signs and the expression levels of NLRP3 inflammasome network proteins in brain parenchyma and body fluids are urgently needed. Research to identify and design compounds and therapeutic strategies to target the NLRP3 inflammasome and modulate the detrimental inflammatory processes without excessively disturbing the immune defense progress may be essential for the treatment of ischemic stroke. Extensive clinical trials are required to develop safer and more effective agents targeting the NLRP3 inflammasome pathway in humans.

Availability of data and materials

Not applicable.

Authors' contributions

FC, XW, CM, SL, SZ, TX made substantial contributions to the conception of the present study and wrote the manuscript and critically reviewed it. XY, YG, CZ, CLe, CLi, SF, HT, YC, QW contributed to searching related articles and reviewing the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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