Therapeutic strategies targeting the NLRP3-mediated inflammatory response and pyroptosis in cerebral ischemia/reperfusion injury (Review)

WAN-LI DUAN[1*], XUE-JIE WANG[1*], YA-PING MA[1*], ZHI-MEI SHENG[1], HAO DONG[1], LI-YING ZHANG[1], BAO-GANG ZHANG[1,2] and MAO-TAO HE[1,2]

[1]Department of Diagnostic Pathology, School of Basic Medical Sciences, Weifang Medical University; [2]Department of Pathology, Affiliated Hospital of Weifang Medical University, Weifang, Shandong 261041, P.R. China

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Abstract. Ischemic stroke poses a major threat to human health. Therefore, the molecular mechanisms of cerebral ischemia/reperfusion injury (CIRI) need to be further clarified, and the associated treatment approaches require exploration. The NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome serves an important role in causing CIRI, and its activation exacerbates the underlying injury. Activation of the NLRP3 inflammasome triggers the maturation and production of the inflammatory molecules IL-1β and IL-18, as well as gasdermin-D-mediated pyroptosis and CIRI damage. Thus, the NLRP3 inflammasome may be a viable target for the treatment of CIRI. In the present review, the mechanisms of the NLRP3 inflammasome in the intense inflammatory response and pyroptosis induced by CIRI are discussed, and the therapeutic strategies that target the NLRP3-mediated inflammatory response and pyroptosis in CIRI are summarized. At present, certain drugs have already been studied, highlighting future therapeutic perspectives.

Correspondence to: Dr Mao-Tao He or Dr Bao-Gang Zhang, Department of Diagnostic Pathology, School of Basic Medical Sciences, Weifang Medical University, 7166 Baotong Street, Weifang, Shandong 261041, P.R. China
E-mail: hemaotao@wfmc.edu.cn
E-mail: zhangbg@wfmc.edu.cn

*Contributed equally

Abbreviations: CIRI, cerebral ischemia/reperfusion injury; NLRP3, NOD-like receptor thermal protein domain associated protein 3; GSDMD, gasdermin-D; ROS, reactive oxygen species; NLR, nucleotide-binding oligomeric domain-like receptor; ASC, apoptosis-associated spot-like protein; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; TLR, toll-like receptor; ADAM8, a disintegrin and metalloproteinase 8; TXNIP, thioredoxin-interacting protein; UCP2, uncoupling protein 2; ERS, endoplasmic reticulum stress; α7nAChR, α7 nicotinic acetylcholine receptor; OGD/R, oxygen-glucose deprivation/recovery; CYLD, cylindromatosis; OA, oleanolic acid; BMSC-Exos, exosomes secreted from bone marrow mesenchymal stem cells; MSC-exos, mesenchymal stem cell-derived exosomes; VNS, vagus nerve stimulation; GLGZG, Gualou Guizhi granule; ICA, icariin

Key words: NLRP3 inflammasome, CIRI, inflammation, pyroptosis, therapeutic target

Contents

1. Introduction

Stroke is a leading cause of human death and disability, and poses a major threat to humans (1). In total, ~85% of stroke are caused by cerebral ischemia and 15% are caused by cerebral hemorrhage (2). Cerebral ischemia is the result of a lack of blood supply due to occlusion of the cerebral arteries, which results in a lack of glucose and oxygen supply to all brain cells. Therefore, lack of blood in the brain disturbs intracellular homeostasis, which causes inflammation, oxidative damage, excitotoxicity and finally the death of brain cells (3). Thrombolysis to restore blood supply to the brain is currently a viable treatment option for (4). However, rapid reperfusion can lead to further damage to areas of the brain, a condition known as cerebral ischemia/reperfusion injury (CIRI) (5,6). Nevertheless, there are a number of possible mechanisms by which CIRI can occur, including inflammatory response (7), Ca^{2+} overload (8), overproduction of reactive oxygen species (ROS) (9), neuronal damage caused by glutamate (10) and mitochondria induced-autophagy (11). Of these mechanisms, neuroinflammation serves a key role in CIRI, including via local cytokine upregulation and leukocyte infiltration (12).

Inflammasomes are protein complexes, and potent substances that activate inflammatory mediators, which was first proposed by Martinon *et al* (13) in 2002. Inflammasomes are part of the innate immune response of the body against pathogen invasion, inflammasomes are activated by cellular infection or stress stimulation and induce the expression, maturation and release of various pro-inflammatory cytokines like IL-18 and IL-1β, thereby triggering a range of inflammatory responses (14,15). Inflammasomes are mainly composed of the nucleotide-binding oligomeric domain-like receptor (NLR) family, which can be divided into three subfamilies: The NLRP, nucleotide-binding oligomerization domains (NODs) and the ice protease-activating factor (IPAF) subfamilies, including NLR family apoptosis inhibitory protein and IPAF (14).

The NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome is the most widely studied inflammasomes and contains NLRP3, Pro-caspase-1 and apoptosis-associated spot-like protein (ASC) (16-18) (Fig. 1B). NLRP3 consists of an amino-terminal pyrin domain structural domain, a central nucleotide-binding structural domain and an oligomeric structural domain (19) (Fig. 1A). NLRP3 inflammasome assembly is initiated by the interaction of the pyrin structural domain of NLRP3 with the pyrin structural domain of ASC (20). The NLRP3 inflammasome serves a key role in the innate immune system, and activation of the NLRP3 inflammasome mediates the activation of downstream caspase-1 and secretion of the pro-inflammatory cytokines, IL-1β and IL-18, in response to microbial invasion and cellular damage (21). The NLRP3 inflammasome can be activated by different stimuli, including damage-associated molecular patterns (DAMPS) and pathogen-associated molecular patterns (PAMPs) (16). DAMPS are regulated by the pro-inflammatory pathway, such as toll-like receptor (TLR)/NF-κB) signaling pathway, which increases NLRP3 and IL-1β protein expression (22,23) and reduces the activation threshold of NLRP3 through additional post-translational modifications (24-26). PAMPs include Ca^{2+} signaling disruption, mitochondrial dysfunction, ROS production, K^+ efflux and lysosomal rupture, promoting assembly of the inflammasome and activating caspase-1, which catalyzes the conversion of pro-IL-1β to active IL-1β (27,28).

As a novel form of programmed cell death, pyroptosis is mainly induced by the gasdermin (GSDM) family (29,30). Of the six members of the GSDM family, five are closely related to pyroptosis and these are GSDMA, GSDMB, GSDMC, GSDMD and GSDME (31). Members of the GSDM family have highly conserved N-terminal and C-terminal domains, and the N-terminal domain can form pores in the cell membrane, causing pyroptosis (32). Activation of inflammasomes can mediate the scission of GSDMD by caspase, which results in formation of GSDMD-N-terminal and finally leads to pyroptosis (33). In addition, pyroptosis is also a crucial pathophysiological process in ischemic stroke (34).

2. NLRP3 inflammasome is involved in CIRI

Activation of the NLRP3 inflammasome can induce CIRI (35). Abulafia *et al* (36) first demonstrated that the NLRP inflammasome serves a key role in the inflammatory response to ischemic stroke. Furthermore, CIRI causes upregulation of NLRP3 expression (37) and inhibition of the NLRP3 inflammasome might exert a neuroprotective effect to attenuate CIRI following stroke onset (38). In addition, the NLRP3 inflammasome inhibitor, MCC950, attenuates cerebral infarction, edema, hemorrhagic transformation and neurological deficits in mice following CIRI (38). Furthermore, NLRP3 inhibition facilitates diabetes-mediated cognitive impairment and vascular neural remodeling after CIRI (39,40). Additionally, the NLRP3 inflammasome drives the inflammatory response in CIRI as revealed in a study by Franke *et al* (35), which demonstrated that NLRP3 mRNA and protein expression was elevated following CIRI, meanwhile other inflammatory vesicles did not change significantly (NLR family CARD domain-containing 4, absent in melanoma 2 and NLRP1). A disintegrin and metalloproteinase 8 (ADAM8) is a transmembrane protein with a number of different functions that serves an important role in tumor and neuroinflammation-related diseases (41). In addition, there is evidence indicating that ADAM8 can aggravate CIRI by activating the NLRP3 inflammasome (41). Furthermore, the NLRP3 inflammasome is a key causative factor in stroke-induced blood-brain barrier disruption, in which the NLRP3 inflammasome exacerbates CIRI by activating inflammatory signaling cascades, inducing pyroptosis of brain endothelial cells and promoting disruption of the blood-brain barrier (42). Early inhibition or blockade of NLRP3 activation protects against CIRI by reducing inflammation and stabilizing the blood-brain barrier (42). In summary, the NLRP3 inflammasome activation is one of the key mechanisms for CIRI. Therefore, further research that focuses on the NLRP3 inflammasome as a therapeutic target for CIRI and the prevention and treatment of ischemic stroke is essential.

3. Mechanisms of the NLRP3 inflammasome in CIRI

ROS-mediated activation of the NLRP3 inflammasome. Oxidative stress is known to be implicated in the pathogenesis of CIRI, and a study has demonstrated that oxidative stress serves an important role in the prevention and treatment of ischemic stroke by regulating the level of inflammation (43). Oxidative stress can produce ROS. ROS are radicals containing oxygen atoms, and include H_2O_2, O^{2-} and OH^-. ROS are mainly derived from the mitochondria and can also be produced by cellular enzymes, including lipoxygenase and cyclooxygenase, which are responsible for inflammasome activation (44). CIRI takes place when the tissue damage caused by restoration of the blood supply to the tissue after a period of ischemia causes tissue damage. This reconstitution of blood flow causes accumulation of ROS, disturbance of cellular ion homeostasis and induce inflammatory response, thereby triggering further damage to ischemic tissues (44). In particular, ROS induce NLRP3 inflammasome activation and stimulate tissue inflammation during CIRI (44,45). Furthermore, ROS have been demonstrated to be a proximal signal for NLRP3 inflammasome activation in inflammatory diseases including CIRI, renal and cardiac ischemia-reperfusion (46). Pro-oxidant and pro-inflammatory thioredoxin-interacting protein (TXNIP), a key regulator of ROS, is associated with inflammation (47). TXNIP is required for NLRP3 activation, which leads to the

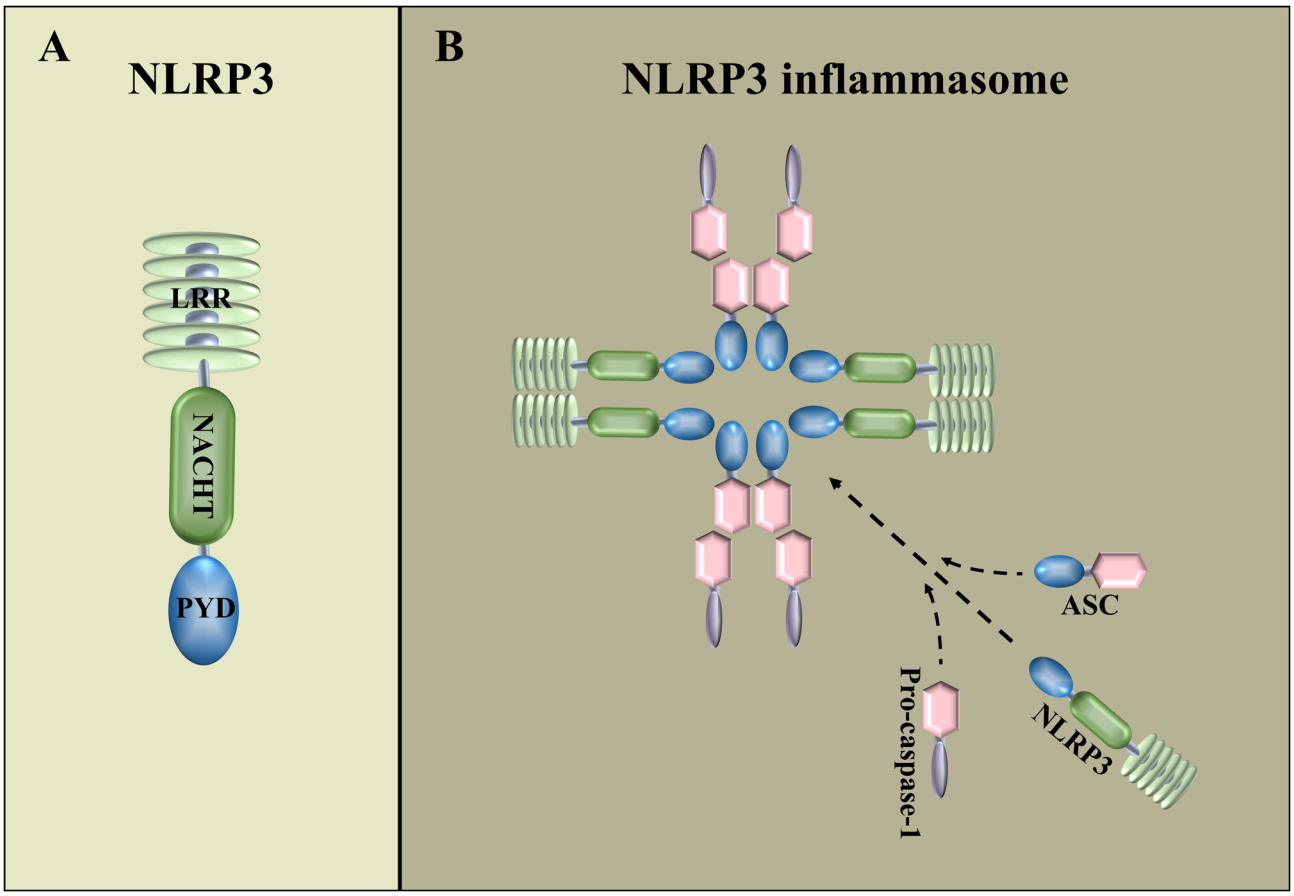

Figure 1. Structure of NLRP3 and the NLRP3 inflammasome. (A) NLRP3 consists of an amino-terminal PYD structural domain, a central nucleotide-binding structural domain and an oligomeric structural domain. (B) The NLRP3 inflammasome contains NLRP3, Pro-caspase-1 and ASC. ASC, apoptosis-associated spot-like protein; NACHT, nucleotide-binding and oligomerization domain; LRR, leucine-rich repeat; NLRP3, NOD-like receptor thermal protein domain associated protein 3; PYD, pyrin domain.

initiation or worsening of the disease state (48). The increase in ROS generation leads to the upregulation of thioredoxin, TXNIP recruitment of NLRP3 and NLRP3 activation (49). TXNIP is activated by ROS and promotes NLRP3 inflammasome activation by binding to NLRP3 following ischemic stroke (Fig. 2), and inhibition of TXNIP expression reduces inflammasome activation after ischemic stroke (49,50). Mitochondria also serve an important role in the regulation of ROS. Uncoupling protein 2 (UCP2) is an inner membrane protein of the mitochondria that has been reported to regulate mitochondrial potential and ROS production (51,52). At present, there is a study has reported that UCP2 serves an important role in CIRI. UCP2 deficiency aggravates hyperglycemia-induced CIRI by enhancing NLRP3 inflammasome activation and ROS generation (53). Since ROS are an important activator of NLRP3 following CIRI, strategies that eliminate excessive ROS may be effective therapeutic approaches for ischemic stroke.

Activation of the TLR4/NF-κB signaling pathway mediates upregulation of NLRP3 inflammasome expression. TLR4 is a transmembrane receptor protein of the innate immune system that is upregulated following CIRI (54). Upregulation of TLR4 activates NF-κB, which induces the release of number of proinflammatory factors such as IL-18 and IL-1β, triggering an inflammatory response and leading to brain injury (54). Microglia are intrinsic myeloid cells of the central nervous system and are involved in the development of CIRI. For macrophages or microglia, the presence of an NLRP3 activator alone is not sufficient to induce inflammasome activation, and its activation requires initiation signals (55). NLRP3 inflammasome activation must first be induced by initiating stimuli, such as ligands for TLRs, NLRs (such as NOD1 and NOD2) or cytokine receptors, which activate the transcription factor NF-κB and upregulate NLRP3 and IL-1β expression (55). Previous studies have demonstrated that activation of the TLR4/NF-κB signaling pathway is a fundamental step in the formation of the NLRP3 inflammasome and is closely associated with activation of the NLRP3 inflammasome (56,57). TLR4 serves an important role in CIRI and is widely expressed in the brain, especially in microglia and endothelial cells (58,59). Furthermore, inhibition of the TLR4/NF-κB signaling pathway can reduce CIRI by regulating the inflammatory response and apoptosis (60). Collectively, the aforementioned studies have demonstrated that TLR4 activation is a key factor in the upregulation of NLRP3 expression following CIRI, implying that targeting TLR4 or its downstream proteins is likely to be an effective treatment for ischemic stroke (Fig. 2).

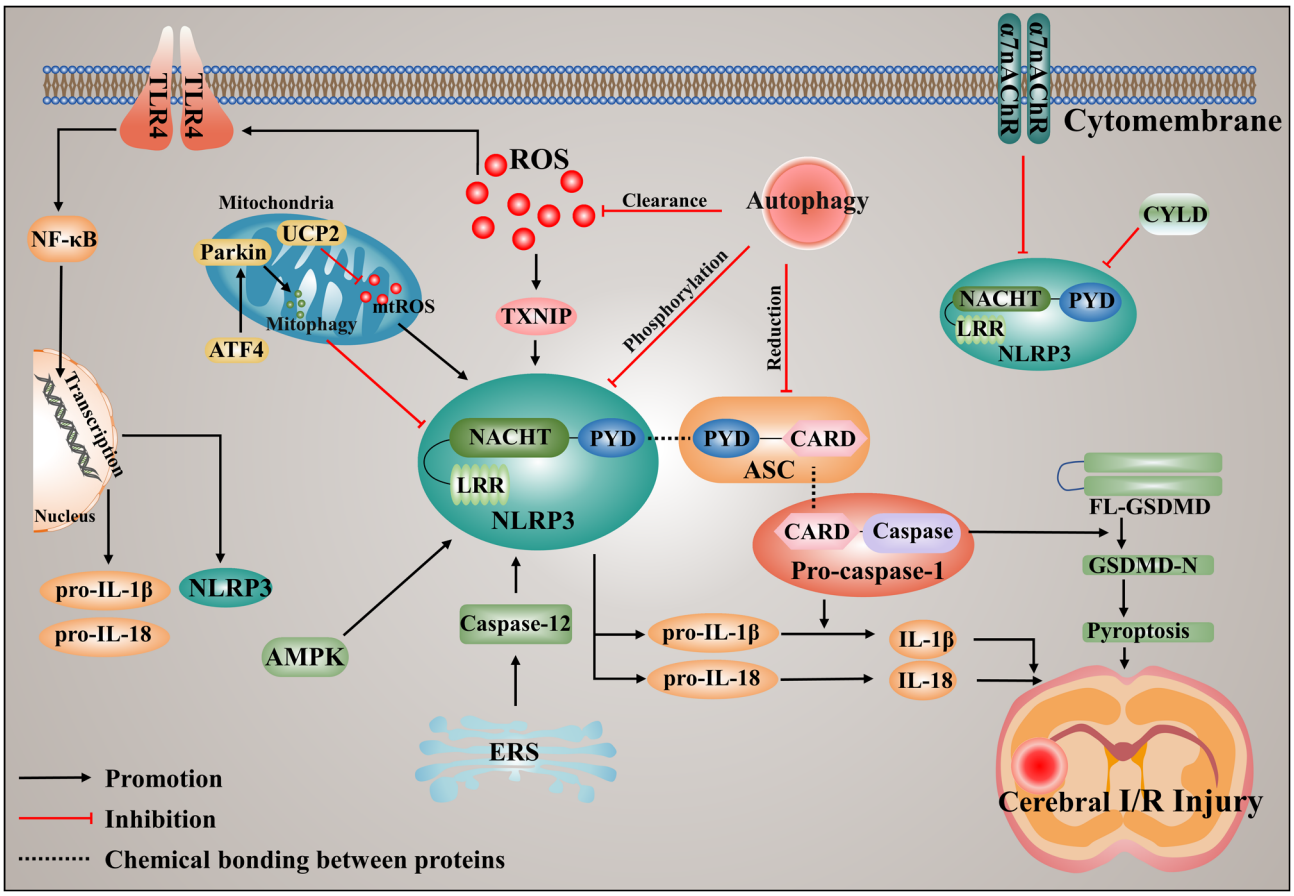

Figure 2. Mechanisms of the NLRP3 inflammasome in CIRI. ROS act as a signal for NLRP3 activation during CIRI. TLR4 triggers NLRP3-mediated inflammatory responses and pyroptosis by increasing NLRP3 expression by regulating the NF-κB transcription factor pathway. Autophagy can prevent NLRP3 activation by eliminating ROS. Additionally, α7nAChR and CYLD activation can inhibit NLRP3. Caspase-12 is responsible for activating the NLRP3 inflammasome. α7nAChR, α7 nicotinic acetylcholine receptor; AMPK, AMP-activated protein kinase; ASC, apoptosis-associated spot-like protein; ATF4, activating transcription factor 4; CIRI, cerebral ischemia/reperfusion injury; CYLD, cylindromatosis; ERS, endoplasmic reticulum stress; LRR, leucine-rich repeat; NLRP3, NOD-like receptor thermal protein domain associated protein 3; PYD, pyrin domain; ROS, reactive oxygen species; TLR4, toll-like receptor 4; TXNIP, thioredoxin-interacting protein; UCP2, uncoupling protein 2; mtROS, mitochondrial reactive oxygen species; NACHT, Nucleotide-binding and oligomerization domain; CARD, caspase activation and recruitment domain; FL-GSDMD, full length-GSDMD; GSDMD-N, GSDMD-N-terminal.

Autophagy can inhibit NLRP3 inflammasome activation. Autophagy acts as a stable self-sustaining process in numerous physiological and pathological processes of eukaryotic cells. In this process, bilayers encapsulate pathogens, abnormal proteins and organelles to form autophagosomes, which are transferred to lysosomes for degradation (61). Autophagy can be classified as macroautophagy, microautophagy and chaperone-mediated autophagy depending on the duration of action, the inducing signal, the type of target and the transit pathway into the lysosome (16,62). Macroautophagy involves the formation of double-membrane vesicles that separate the cytoplasm. These intact vesicles, termed autophagic vesicles, then fuse with lysosomes for subsequent degradation (63,64). In microautophagy, the material to be degraded reaches the lysosomal lumen via lysosomal invagination or the endoplasmic membrane (65,66). Chaperone-mediated autophagy only occurs in mammalian cells and allows for the selective degradation of proteins with specific amino acid sequences (67). Among these three autophagic processes, macroautophagy, commonly termed autophagy, is the most active form and has been extensively studied in disease (68,69). Conserved proteins such as Beclin1, LC3 and P62 are involved in the autophagic process and are considered autophagy-related proteins (63). Autophagy is affected by various parameters such as endoplasmic reticulum stress (ERS), ROS, nutritional deficiencies, immune or inflammatory stimuli, accumulation of organelle damage, and the Ca^{2+} concentration (70,71). Under physiological conditions, autophagy is typically maintained at basal levels. However, in pathological states, upregulated autophagy removes dysfunctional proteins from cells and aids cell survival (72). Autophagy can inhibit NLRP3 activation by reducing ASC expression, increasing phosphorylation of NLRP3 and scavenging ROS (16). The cytoplasmic protein, activating transcription factor 4 (ATF4), serves an important role in the regulation of autophagy, and ATF4 is a member of the activating transcription factor/cAMP response element binding protein family (73). As a transcription factor, ATF4 was involved in Endoplasmic reticulum (ER) homeostasis, autophagy and inflammation response (73). In addition, ATF4 inhibits the NLRP3 inflammasome-mediated inflammatory response via upregulation of Parkin-dependent mitochondrial autophagy in CIRI (74). Finally, autophagy can target the degradation of IL-1β, inhibit activation of the NLRP3 inflammasome and reduce the release of inflammatory cytokines (75,76). Thus,

autophagy has been shown to negatively regulate the NLRP3 inflammasome activation and effectively reduce CIRI (Fig. 2).

Other pathways mediating the inhibition of the NLRP3 inflammasome. In addition to the aforementioned three methods of activation, NLRP3 can also be activated by other pathways following CIRI. For example, there is evidence that the α7 nicotinic acetylcholine receptor (α7nAChR) is critical in mediating cholinergic anti-inflammatory signaling (77). Electroacupuncture promotes α7nAChR-mediated inhibition of the NLRP3 inflammasome, thereby reducing CIRI and neuroinflammation (78), which implies that α7nAChR may be an upstream signal for NLRP3 activation. ERS is severe in ischemic brain injury and leads to an inflammatory response via activation of caspase-12 (79). In a previous study, pretreatment with the caspase-12 specific inhibitor Z-ATAD-FMK attenuated cell injury and apoptosis, and reduced the levels of NLRP3, caspase-1, IL-1β and cleaved caspase-3 compared with oxygen-glucose deprivation/recovery (OGD/R) group (79). Therefore, the NLRP3 inflammasome signaling pathway may be inhibited by suppression of caspase-12 signaling to attenuate CIRI. In addition, electroacupuncture induces upregulation of neuronal cylindromatosis (CYLD) expression, which exerts anti-inflammatory and neuroprotective effects by inhibiting NLRP3 expression, regulates the interaction between neurons and microglia, reduces M1 microglia in the peri-ischemic cortex, and improves the activation of M2 microglia, thereby reducing CIRI (80). Collectively, the above studies demonstrated that both α7nAChR and CYLD can inhibit NLRP3 inflammasome activation, while ERS-mediated caspase-12 activation can upregulate NLRP3 expression (Fig. 2).

Activation of the NLRP3 inflammasome promotes the release of downstream inflammatory factors and facilitates pyroptosis in CIRI. Activation of inflammasomes has been associated with various inflammatory diseases, including post-ischemic inflammation following ischemic stroke (12). Inflammasomes mediate the activation of caspase-1, which in turn induces the secretion of pro-inflammatory cytokines and pyroptosis (81). Caspase-1 is activated upon recruitment to the inflammasome, then activated caspase-1 cleaves the cytokines Pro-IL-1β and Pro-IL-18 into their mature bioactive forms (13,82). IL-1β controls fever, pain threshold, vasodilation, and hypotension, and promotes immune cell infiltration into infected or damaged tissues (83). IL-18 is required for production of IFN-γ, a costimulatory cytokine that mediates adaptive immunity (84). CIRI activates NLRP3, induces the release of IL-1β and IL-18 and promotes maturation of GSDMD-N, and leads to severe neuronal pyroptosis (85). Previous studies have demonstrated that the expression levels of GSDMD-N, NLRP1/3, IL-1β and IL-18 in Sprague-Dawley rats and mice were increased following CIRI compared with the Sham group, and intervention treatment of these inflammatory factors attenuated CIRI (86-90). In another study, the mRNA expression levels of NLRP3, caspase-1, IL-1β, IL-6 and TNF-α were increased in microglia after OGD/R treatment compared with the control group (91). Overall, NLRP3 inflammasome activation promotes the release of downstream inflammatory factors and causes GSDMD-mediated pyroptosis following CIRI (Fig. 2).

4. Therapeutic strategies targeting NLRP3 in CIRI

Therapeutic strategies that alleviate CIRI by reducing the activation of NLRP3 via ROS inhibition. During CIRI, ROS stimulate tissue inflammation and activate the NLRP3 inflammasome. Inflammatory diseases are often characterized by the activation of the NLRP3 inflammasome, which is primarily triggered by ROS. Therefore, inhibiting the production of ROS or increasing their consumption following CIRI could be a viable treatment option for stroke (92). In a study by Cao *et al* (93), it was demonstrated that ruscogenin reduced ROS levels following CIRI, which in turn inhibited TXNIP/NLRP3 inflammasome activation and mitigated ischemia-induced blood-brain barrier dysfunction. Additionally, astilbin has been reported to reduce the brain infarct volume and alleviate neurological deficits in middle cerebral artery occlusion (MCAO) rats (94). Furthermore, astilbin has been demonstrated to inhibit cellular inflammation induced by OGD/R by suppressing the activation of the ROS-NLRP3 inflammasome axis (94). Cepharanthine has also been demonstrated to reduce CIRI by inhibiting the 12/15-lipoxygenase signaling pathway, leading to a decrease in ROS and the downregulation of NLRP3 expression (95). In addition, ATN-161 has been indicated to exert a protective effect on cells by reducing the levels of mitochondrial superoxide radicals, thereby alleviating oxidative stress and intracellular ROS during the onset of CIRI (96). However, tomentosin promotes the production of superoxide dismutase in rats during CIRI, which scavenges free radicals, accelerates the antioxidant system, inhibits NLRP3 signaling and attenuates CIRI (97). Oleanolic acid (OA) has been demonstrated to reduce microglia activation and ROS in CIRI, suggesting that OA may exert neuroprotective effects on ischemic stroke by inhibiting NLRP3 inflammasome activation through the reduction of ROS (98). The aforementioned studies have demonstrated that decreasing ROS levels can mitigate the harm caused by CIRI or cellular OGD/R treatment. Therefore, inhibiting the ROS may be a viable option for the treatment of stroke (Table I).

Therapeutic strategies that attenuate CIRI by inhibiting TRL4-mediated NLRP3 upregulation. TLR4 is an important factor in CIRI, and its downstream NF-κB signaling pathway is crucial in the formation of the NLRP3 inflammasome and is closely linked to its activation (54). Inhibiting the TLR4/NF-κB signaling pathway at the onset of CIRI may be a viable treatment option for stroke (54). Cui *et al* (99) conducted a study on anthocyanin derived from *Myrica rubra*, and revealed that treatment of ischemia/reperfusion (I/R) mice with purified anthocyanin extracts for 1 week resulted in a decrease in brain infarct volume, disease damage, and nitric oxide and malondialdehyde levels, while superoxide dismutase levels were increased compared with Sham group (99). In addition, treatment with meisoindigo resulted in improvements in neurological scores, reduced infarct volume and decreased brain edema in MCAO/R mice compared with Sham group. Further analysis revealed that meisoindigo inhibited the expression of TLR4/NF-κB signaling pathway-related proteins in a dose-dependent manner. This inhibition led to the downregulation of NLRP3, high mobility group box 1 and IL-1β expression (100). D-carvone has been reported to

Table I. Therapeutic strategies using the NLRP3 inflammasome as a target in cerebral ischemia/reperfusion.

First author/s, year	Drug or treatment	Manner of NLRP3 inhibition	Mode of action	(Refs.)
Cao G, 2016	Ruscogenin	Reduces ROS	Reduces ROS levels	(93)
Li Y, 2020	Astilbin		Inhibits ROS-NLRP3 axis	(94)
Zhao J, 2020	Cepharanthine		Inhibits 12/15-lox signaling pathway	(95)
Amruta N, 2021	ATN-161		Reduces mtROS and intracellular ROS	(96)
He J, 2020	Tomentosin		Enhances antioxidant system	(97)
Sapkota A, 2022	Oleanolic acid		Attenuates ROS	(98)
Cui HX, 2018	PAES	Inhibits TLR4/NF-κB signaling pathway	Inhibits the TLR4/NF-κB/NLRP3 signaling pathway	(99)
Ye Y, 2019	Meisoindigo		Inhibits the TLR4/NF-κB signaling pathway	(100)
Dai M, 2020	D-carvone		Inhibits the TLR4 signaling pathway	(101)
Wang K, 2020	Exosomes treated with melatonin		Modulates the TLR4/NF-κB signaling pathway	(102)
He J, 2020	Tomentosin		Reduces TLR4 expression	(97)
Han D, 2020	Vinpocetine		Inhibits the NF-κB signaling pathway	(103)
Liu J, 2021	Salidroside		Inhibits TLR4/NF-κB signaling pathway	(104)
Ran Y, 2021	Curcumin		Inhibits the NF-κB/NLRP3 signaling pathway	(105)
Zeng *et al*, 2020	BMSC-Exos	Enhances autophagy-mediated inhibition of NLRP3 activation	Promotes the AMPK/mTOR signaling pathway	(107)
Hu Z, 2021	MSC-exos		Upregulates FOXO3a expression	(108)
Huang Z, 2021	Pien-Tze-Huang		Promotes the AMPK/mTOR/ULK1 signaling pathway	(109)
Ma C, 2019	Qingkailing	Other pathway	Inhibit AMPK-mediated NLRP3 activation	(110)
Xiao L, 2021	AST IV		Activates Nrf2	(111)
Jiang T, 2019 & Lin X, 2021	Electroacupuncture		Promotes α7nAChR and CYLD-mediated inhibition of NLRP3	(78,80)
Tang H, 2022	VNS		Promotes inhibition of NLRP3 by α7nAChR	(112)
Liu L, 2020	Z-ATAD-FMK		Inhibits caspase-12	(79)
Zhang Y, 2021	GLGZG		Activates the PI3K/AKT signaling pathway	(113)
Wang B, 2021	Tongxinluo		Reduces NLRP3 expression	(114)
Mo ZT, 2021	ICA		Inhibits the IRE1/XBP1s signaling pathway	(91)
Shi M, 2022	Remimazolam		Downregulates NLRP3 expression	(89)
Zhu *et al*, 2022	Xingxiong injection		Activates the AKT/Nrf2 signaling pathway	(90)

α7nAChR, α7 nicotinic acetylcholine receptor; 12/15-lox, 12/15-lipoxygenase; AMPK, AMP-activated protein kinase; AST IV, astragaloside IV; BMSC-Exos, exosomes secreted from bone marrow mesenchymal stem cells; CYLD, cylindromatosis; GLGZG, Gualou Guizhi granule; ICA, icariin; IRE1, inositol-requiring enzyme type 1; MSC-exos, mesenchymal stem cell-derived exosomes; NLRP3, NOD-like receptor thermal protein domain associated protein 3; Nrf2, nuclear factor erythroid 2-related factor 2; PAES, purified anthocyanin extracts; ROS, reactive oxygen species; TLR4, toll-like receptor 4; VNS, vagus nerve stimulation; XBP1s, spliced form of X-box binding protein 1; mtROS, mitochondrial reactive oxygen species; ULK1, UNC-51-like kinase 1.

inhibit the TLR4-induced signaling pathway of inflammatory cytokines and reduce NLRP3 expression, leading to the successful amelioration of I/R-induced neuroinflammation in the brains of rats. As a result, I/R-induced brain injury in the hippocampal and cortical regions was attenuated (101). Exosomes treated with melatonin have been shown to effectively reduce the infarct size and improve functional recovery by modulating the TLR4/NF-κB signaling pathway and reducing NLRP3-induced inflammation following CIRI (102). Additionally, tomentosin treatment enhances antioxidant capacity to reduce ROS levels, while also reducing the expression of TLR4 and its downstream pro-inflammatory cytokines. This ultimately inhibits NLRP3 expression and attenuates CIRI (97). Vinpocetine has been revealed to inhibit the NF-κB pathway-related proteins, which in turn downregulates NLRP3 expression levels. This inhibition leads to a reduction in the release of pro-inflammatory cytokines, resulting in a decrease in the size of cerebral infarcts and an improvement in behavioral recovery in MCAO mice (103). Salidroside has been demonstrated to reverse NLRP3 inflammasome activation, resulting in downregulated levels of NLRP3, ASC, caspase-1, IL-1β and IL-18 proteins, as well as the suppression of key components of the TLR4 signaling pathway in BV2 cells following OGD/R (104). The specific TLR4 inhibitor, TAK242, exhibited the same effect as salidroside on BV2 cells following OGD/R induction, indicating that salidroside has the capability to specifically inhibit the TLR4/NF-κB signaling pathway, reducing NLRP3 expression and attenuating CIRI (104). Curcumin has been demonstrated to attenuate white matter damage caused by stroke to some extent by inhibiting the NF-κB/NLRP3 signaling pathway, improving functional outcomes and reducing microglia apoptosis (105). In summary, the aforementioned studies demonstrated that inhibiting the TLR4/NF-κB signaling pathway through pharmacological treatment can effectively suppress the expression and activation of NLRP3, thereby reducing the inflammatory response and cellular damage caused by CIRI. Furthermore, inhibiting the upregulation of NLRP3 expression mediated by TLR4 may be a viable clinical treatment option for stroke (Table I).

Therapeutic strategies that mitigate CIRI by enhancing the autophagy-mediated inhibition of NLRP3 activation. Autophagy serves a crucial role in various pathophysiological processes such as renal and cardiac ischemia-reperfusion and CIRI. In pathological conditions, autophagy can hinder the activation of the NLRP3 inflammasome by eliminating endogenous inflammasome activators such as ROS, cytokines and damaged mitochondria from inflammatory components. Inducing cellular autophagy through pharmacological intervention during the onset of CIRI may be a viable option for treating patients following ischemic stroke (106). Exosomes secreted from bone marrow mesenchymal stem cells (BMSC-Exos) have been found to increase autophagic flux in PC12 cells treated with OGD/R, while also inhibiting OGD/R-induced pyroptosis (107). Experimental data further indicated that BMSC-Exos treatment led to decreased NLRP3 expression, as well as elevated LC3 II/I and phosphorylated-AMPK)/AMPK levels (107). These findings suggested that BMSC-Exos promoted autophagic flux in PC12 cells via the AMPK/mTOR signaling pathway, while also inhibiting NLRP3 inflammasome-mediated pyroptosis (107). As a result, BMSC-Exos offer protective benefits to PC12 cells, shielding the cells from OGD/R injury (107). In a similar study, it was identified that human umbilical cord mesenchymal stem cell-derived exosomes (MSC-Exos) had a positive impact on BV2 cell viability following OGD/R (108). Additionally, the expression levels of NLRP3, cleaved caspase-1 and GSDMD-N, as well as the release of IL-1β and IL-18, were decreased, while translocase of outer mitochondrial membrane 20 and cytochrome c oxidase subunit 4 isoform 1 expression was increased. However, the neuroprotective effect of MSC-Exos was partially abolished by FOXO3a small interfering RNA treatment, which also attenuated the inhibition of mitochondrial phagocytosis and pyroptosis induced by MSC-Exos treatment. This study suggests that FOXO3a expression is increased by MSC-exos, which in turn enhances mitochondrial autophagy in microglia. MSC-Exos treatment inhibits pyroptosis induced by CIRI and ultimately reduces nerve damage (108). Pien-Tze-Huang has been demonstrated to regulate essential autophagic proteins via the AMPK/mTOR/unc-51 like autophagy activating kinase 1 (ULK1)-related signaling pathway. This regulation enhances the autophagic response and inhibits the production of key pro-inflammatory mediators, as well as the expression of NLRP3 and caspase-1 p20 proteins in lipopolysaccharide-induced BV2 cells. These findings suggest that Pien-Tze-Huang may enhance autophagy following CIRI via the AMPK/mTOR/ULK1 signaling pathway, thereby reducing NLRP3-associated neuroinflammation (109). In summary, the aforementioned studies all indicated that activation of the NLRP3 inflammasome can be effectively suppressed by enhancing autophagy-mediated inhibition of NLRP3 activation. This in turn can lead to a protection in CIRI (Table I).

Therapeutic strategies that attenuate CIRI by inhibiting NLRP3 activation or expression through other pathways. In addition to inhibiting the activation of the NLRP3 inflammasome by reducing ROS, regulating the TLR4/NF-κB pathway or enhancing autophagy, there are a number of other therapeutic strategies available to inhibit NLRP3 through different signaling pathways (Table I). The NLRP3 inflammasome serves a crucial role in regulating the release of inflammatory factors and GSDMD-mediated pyroptosis in CIRI. Inhibiting NLRP3 activation or expression can effectively reduce the injury caused by CIRI (18). It has been demonstrated that Qingkailing can effectively reduce the inflammatory response following CIRI, which is achieved by inhibiting AMPK-mediated NLRP3 activation and in turn attenuating CIRI (110). Similarly, astragaloside IV has been demonstrated to alleviate CIRI by inhibiting NLRP3 inflammasome-mediated apoptosis through the activation of nuclear factor erythroid 2-related factor 2 (Nrf2) (111). Additionally, electroacupuncture has been demonstrated to promote α7nAChR and CYLD-mediated inhibition of the NLRP3 inflammasome, thereby reducing CIRI and neuroinflammation (78,80). The use of vagus nerve stimulation (VNS) treatment has been found to inhibit expression of pyroptosis-related molecules, as well as reduce the number of pyrogenic cells and membrane pores. Notably, α7nAChR agonists have been found to

mimic the neuroprotective effects of VNS, which suggests that VNS serves a protective role in CIRI by promoting α7nAChR inhibition of NLRP3-mediated pyroptosis (112). The caspase-12-specific inhibitor, Z-ATAD-FMK, has been reported to reduce cell injury and apoptosis in an OGD/R treatment group by inhibiting the activation of NLRP3. This inhibition also resulted in decreased levels of caspase-1, IL-1β and cleaved caspase-3 compared with control group, indicating that CIRI could be alleviated by inhibiting caspase-12 (79). Gualou Guizhi granule (GLGZG) has been found to effectively reduce CIRI by activating the PI3K/AKT signaling pathway and inhibiting cellular pyroptosis. Additionally, GLGZG suppresses NLRP3 expression and the release of its downstream inflammatory factors (113). Another study found that Tongxinluo can inhibit the pyroptosis of astrocytes during the onset of CIRI. Furthermore, Tongxinluo reduces the expression of NLRP3, caspase-11/1, IL-1β and IL6, and attenuates CIRI by decreasing the accumulation of amyloid-β peptide (114). Icariin has been demonstrated to reduce NLRP3 expression by inhibiting the inositol-requiring enzyme 1/X-box binding protein 1 signaling pathway, which decreases the expression of downstream inflammatory factors, reducing pyroptosis and attenuating CIRI (91). In addition, remimazolam has been reported to downregulate the expression of NLRP3 and its associated released inflammatory factors IL-18 and IL-1β, as well as GSDMD, in MCAO rats. This suggests that remimazolam may serve a protective role against CIRI by inhibiting NLRP3 (89). Similarly, Xingxiong injection administration has been demonstrated to activate the AKT/Nrf2 signaling pathway and inhibit the NLRP3 inflammasome during the onset of CIRI, thereby exerting a protective effect (90).

5. Conclusion

Activation of the NLRP3 inflammasome is critical for the mechanisms of CIRI. In the present review, the mechanisms of NLRP3 activation during the onset of CIRI are discussed and are shown in Fig. 2. ROS and TLR4 can promote activation of the NLRP3 inflammasome and its downstream inflammatory response. To some extent, autophagy can negatively regulate NLRP3 activation, which has protected CIRI. Additionally, α7nAChR and CYLD activation can inhibit NLRP3, while caspase-12 activates the NLRP3 inflammasome. Activation of NLRP3 ultimately leads to an inflammatory response, as well as GSDMD-mediated pyroptosis. Furthermore, in the studies described previously have demonstrated that specifically inhibiting the NLRP3 inflammasome can mitigate neuroinflammation and improve outcomes following CIRI. The present review also examines current therapeutic approaches that aim to inhibit the NLRP3 inflammasome to reduce the inflammatory response and pyroptosis during the onset of CIRI (Table I). As such, the present review offers a thorough theoretical foundation for conducting fundamental research on CIRI. Specifically, it provides a detailed overview of the mechanism of action of the NLRP3 inflammasome during CIRI, which will serve as a basis for future research in this field. It is recommended that further research also investigates the role of the NLRP3 inflammasome in pathogenesis and identifies novel therapies. The NLRP3 inflammasome may be considered a crucial target for the treatment of CIRI and may broaden the therapeutic field of ischemic stroke.

To the best of our knowledge, the present review was the first to categorize drugs that serve a protective role in CIRI by targeting the NLRP3 inflammasome with different molecular mechanisms. This provides novel strategies for the clinical treatment of ischemic stroke as well as novel ideas for other diseases in which the NLRP3 inflammasome serves a critical role in the pathologic process. For example, Pien-Tze-Huang is an herbal medicine used for a variety of inflammatory diseases, whether Pien-Tze-Huang has a protective effect in hemorrhagic stroke or in renal ischemic reperfusion is also a question that deserves in-depth exploration. Exploring whether drugs that are protective in CIRI by targeting NLRP3 inflammasome also exert protective roles in other inflammatory diseases will contribute to the greater social value and economic benefits.

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Authors' contributions

WLD, XJW and MTH conceived the study. WLD, YPM, ZMS and HD were involved in literature search, data collection and writing. LYZ, BGZ and MTH reviewed and edited the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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