Neurology Asia 2026; 31(3) : 861 – 878

REVIEW ARTICLES

Inflammatory pathways in cerebral ischemia-reperfusion injury: Focus on the NLRP3 inflammasome

*[1]Yan Cheng, *[3]Ji Wang, [2]Zhengjie Fan, [4]Man He, [5,6]Jing Chen, [5,7]Yiwei Xu
*Y Cheng and J Wang contributed equally to this work and are co-first authors

[1]College of Basic Medical Sciences, China Three Gorges University, Yichang, PR China; [2]Department of Emergency, Yichang Central People's Hospital, The First College of Clinical Medical Science, China Three Gorges University, Yichang, PR China; [3]Department of Emergency and Trauma Surgery, Yichang Central People's Hospital, The First College of Clinical Medical Science, China Three Gorges University, Yichang, PR China; [4]College of Basic Medical Sciences, China Three Gorges University, Yichang, PR China; [5]College of Medicine and Health Sciences, China Three Gorges University, Yichang, PR China; [6]Third-grade Pharmacological Laboratory on Traditional Chinese Medicine, State Administration of Traditional Chinese Medicine, China Three Gorges University, Yichang, PR China; [7]Key Laboratory of Advanced Pharmaceutics, Yichang, PR China

Abstract

Cerebral ischemia-reperfusion injury (CIRI) is a severe neurological disorder characterized by exacerbated brain tissue damage and deteriorated neurological function following the restoration of blood flow after ischemia. The NLRP3 inflammasome, a multi-protein assembly triggered by diverse danger-associated signals, is critically involved in the inflammatory processes underlying CIRI. Its triggering prompts the secretion of IL-1β and IL-18, which in turn amplifies inflammatory reactions, promote neuronal apoptosis and necrosis, disrupt the blood-brain barrier, and impair neurological recovery. This review systematically examines the NLRP3 inflammasome's structural architecture, activation mechanisms, and regulatory pathways, and discusses its close association with CIRI. Additionally, current intervention strategies targeting the NLRP3 inflammasome, including pharmacological inhibitors and traditional Chinese medicine, are summarized, along with future research directions for developing novel treatments and personalized therapies. Elucidating the detailed molecular mechanisms of the NLRP3 inflammasome in CIRI is critical for enhancing therapeutic efficacy and patient prognosis.

Keywords: Cerebral ischemia-reperfusion injury; NLRP3 inflammasome; Inflammation; Neuroinflammation; Pyroptosis; Blood-brain barrier

INTRODUCTION

Cerebral ischemia is a severe neurological disorder that poses a significant threat to human health, and is associated with alarmingly high rates of disability and death, making it a critical public health concern. As shown in Figure 1, according to the World Health Organization[1] between 1990 and 2021, there was a significant increase in the burden of stroke, with a 70.0% increase in the number of new stroke cases, a 44.0% increase in stroke-related deaths, an 86.0% increase in the prevalence of stroke, and a 32% increase in disability-adjusted life years (DALYs) lost due to stroke. After cerebral ischemia occurs, timely restoration of blood perfusion is the key therapeutic strategy to salvage the ischemic brain tissue. However, following restoration of blood flow, some

Address correspondence to: Jing Chen, Ph.D. College of Medicine and Health Sciences, China Three Gorges University, 183, Yi-Ling-Da-Dao, Yichang, 443000,Hubei, China. E-mail: chenjing@ctgu.edu.cn; Yiwei Xu, College of Medicine and Health Sciences, China Three Gorges University, 183, Yi-Ling-Da-Dao, Yichang, 443000,Hubei, China. E-mail: xuyiwei@ctgu.edu.cn

Date of Submission: 9 December 2025; Date of Acceptance: 1 March 2026

https://doi.org/10.54029/2026ixe

patients may experience cerebral ischemia-reperfusion injury (CIRI), which manifests as exacerbated brain tissue damage and deteriorated neurological dysfunction, severely affecting patient prognosis.[2,3] The figure intuitively reflects the growing public health threat posed by stroke, and notably, CIRI as a major complication of ischemic stroke contributes substantially to these adverse outcomes (Figure 1).

Inflammation plays an important role in the development of CIRI. Nucleotide-binding domain, Leucine-rich repeat, and Pyrin domain containing 3 (NLRP3) inflammasome is a master regulator of inflammation, as per recent evidence. Previous studies have focused on the role of the NLRP3 inflammasome in CIRI.[4] The structure of the NLRP3 inflammasome consists of three important components: NLRP3, apoptosis-

Annual Trends in Stroke Data (1990–2021)

Year	New Stroke Cases (10,000)	Stroke-related Deaths (10,000)	Stroke Prevalence (10,000)	Stroke-related DALYs (million years)
1990	717.65	486.11	504..30	53.30
1991	735.98	489.35	517.31	53.93
1992	749.88	498.34	530.43	54.55
1993	764.76	499.65	542.61	55.22
1994	775.40	505.10	555.62	55.91
1995	789.11	510.08	566.11	56.64
1996	797.33	515.89	573.60	57.14
1997	808.48	525.56	587.73	58.04
1998	824.20	531.90	606.42	58.41
1999	837.81	535.32	620.33	58.95
2000	852.80	539.14	632.32	59.24
2001	868.47	546.01	644.60	59.33
2002	888.58	556.19	657.35	60.17
2003	901.34	561.71	675.09	60.63
2004	913.89	568.44	690.66	61.16
2005	936.12	579.76	701.16	61.99
2006	944.13	586.47	704.94	62.63
2007	949.43	595.84	720.44	63.39
2008	962.33	600.54	734.00	64.13
2009	979.86	604.73	750.33	64.96
2010	998.43	613.84	774.52	65.79
2011	1018.29	622.26	794.86	66.63
2012	1024.02	628.58	816.83	67.35
2013	1040.93	632.64	837.48	68.07
2014	1060.46	640.83	856.80	68.45
2015	1079.84	647.19	874.21	69.12
2016	1091.79	657.05	891.98	69.85
2017	1108.31	668.20	914.08	70.70
2018	1120.60	676.50	932.67	71.81
2019	1141.50	684.50	953.12	72.64
2020	1155.73	693.98	964.11	72.98
2021	1169.46	706.43	980.07	73.71

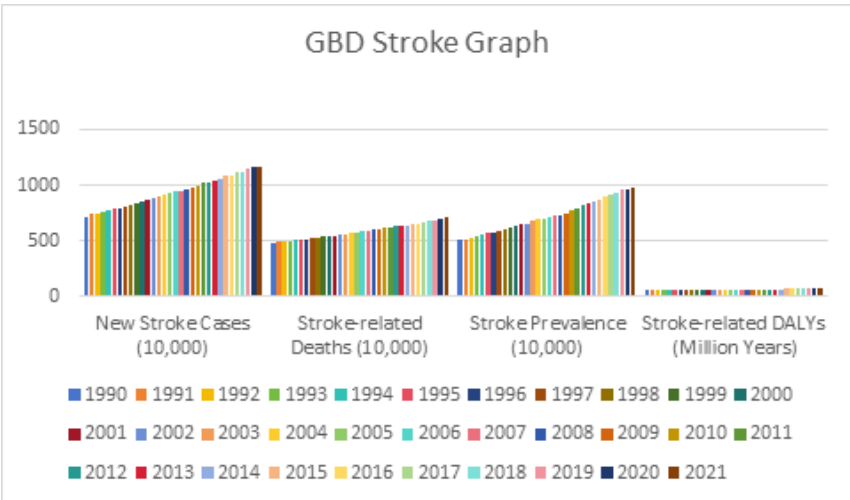

Figure 1. Global burden of stroke (1990-2021). This figure shows the increase in new stroke cases, stroke-related deaths, prevalence, and disability-adjusted life years (DALYs) over the past three decades, based on data from the World Health Organization (2021).

associated speck-like protein containing a caspase recruitment domain (ASC), and pro-caspase-1.[5] Its activation is induced by a range of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs).[6] The cleavage of pro-caspase-1 by the activated NLRP3 inflammasome leads to the release of active caspase-1, which further aids in the cleavage and release of the important pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), causing inflammation.[7] Numerous studies have shown that NLRP3 inflammasome is aberrantly activated in CIRI. When activated in excess, brain tissue damage is aggravated, inflammatory reactions cause neuronal apoptotic and necrotic death, blood-brain barrier deviation occurs, and recovery of neurological function is impaired.[4,8] Consequently, it will be very useful to outline the mechanistic involvement of the NLRP3 inflammasome in CIRI. This can help to understand the disease and lead to clinical intervention, identification of effective targets, and prognosis of patients.

METHODS

This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Literatures search

A comprehensive literature search was performed across PubMed, Web of Science, Embase, CNKI, and Wanfang Data from January 2000 to June 2025. The core search terms included "NLRP3 inflammasome", "cerebral ischemia-reperfusion injury (CIRI)", "neuroinflammation", "pyroptosis", and their corresponding Chinese equivalents.

Eligibility criteria

Studies were included if they focused on the NLRP3 inflammasome in CIRI, including original experimental research (in vitro/in vivo) and peer-reviewed reviews. Exclusion criteria were conference abstracts, non-English/non-Chinese articles, studies lacking NLRP3-specific analysis, and research unrelated to CIRI.

Screening and quality assessment

Two independent investigators screened titles/abstracts and full texts. Discrepancies were resolved by a third investigator. Included in vivo studies were assessed using the ARRIVE guidelines, and in vitro studies were evaluated based on experimental controls and result reproducibility.

OVERVIEW OF CEREBRAL ISCHEMIA-REPERFUSION INJURY

Definition and clinical status

CIRI describes the pathological process by which brain tissue damage is exacerbated and neurological function deteriorates after blood flow is restored following cerebral ischemia.[9] In clinical practice, acute ischemic stroke is the primary cause of CIRI, with its global incidence on the rise.[10,11] China has a high incidence of stroke, with approximately 2 million new cases each year, of which approximately 80% are ischemic strokes. During the treatment of patients with ischemic stroke with thrombolysis, thrombectomy, or other vascular recanalization therapies, cerebral ischemia-reperfusion injury is a common complication that severely affects patient prognosis and quality of life.[12] Statistics indicate that approximately 12% to 16.9% of patients receiving recanalization therapy experience varying degrees of CIRI[13], which increases mortality and disability rates[14] and imposing a heavy burden on families and society (Figure 2). This figure illustrates the progressive pathological process of ischemic stroke, from initial vascular occlusion to CIRI development, highlighting key cascades including oxidative stress, calcium overload, and leukocyte infiltration.

Analysis of pathogenesis

No-reflow phenomenon

After blood flow is restored following cerebral ischemia, some ischemic tissues do not receive effective reperfusion but remain in a state of ischemia with further aggravated damage.[15] This is mainly due to swelling of the nerve and endothelial cells, which narrows or even occludes the microvascular lumen. Simultaneously, the aggregation of white blood cells, platelet adhesion, and activation within the microvasculature form microthrombi, further obstructing microcirculation and preventing normal blood supply to ischemic tissues, thereby exacerbating ischemic-hypoxic injury.[16]

Calcium overload

Ischemic conditions induce neuronal membrane destabilization, resulting in altered permeability. Calcium channels open, allowing a large influx of extracellular Ca^{2+} into cells along a concentration gradient, resulting in an intracellular calcium overload.[17] Intracellular calcium overload can activate various calcium-dependent enzymes such as phospholipases, proteases, and endonucleases. Overactivation of these enzymes results in breakdown of cell membrane phospholipids, disruption of the cytoskeleton, and decomposition of nucleic

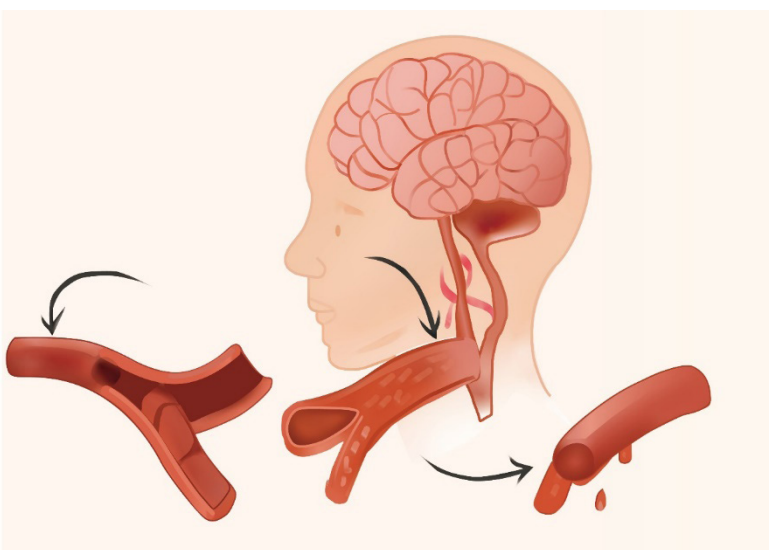

Figure 2. Illustration of ischemic stroke progression and ischemia-reperfusion injury.

acids, ultimately leading to necrosis.[18] Moreover, calcium overload can cause mitochondria to take up excessive Ca^{2+}, causing mitochondrial malfunction, diminished ATP synthesis, and further aggravating problems in cellular energy metabolism.[19]

Oxidative stress

During cerebral ischemia-reperfusion, a sudden increase in oxygen supply triggers a series of redox reactions, generating excessive reactive oxygen species (ROS) including superoxide anions (O_2^-) and hydroxyl radicals ($OH\cdot$).[20] These highly reactive species mediate oxidative damage to critical biomolecules, including membrane lipids, structural proteins, and genetic materials. ROS initiates lipid peroxidation cascades in membrane phospholipids, compromising structural integrity and barrier function, which subsequently elevates permeability and facilitates cytoplasmic content leakage.[21,22] They can also cause protein molecules to cross-link and denature, losing their normal physiological functions.[23] Additionally, free radicals can damage DNA, leading to gene mutations and cell apoptosis. The capacity of ischemic brain tissue to synthesize antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), is diminished, further exacerbating the damage caused by free radicals to the tissue.[24,25]

Energy metabolism disorder

During cerebral ischemia, owing to the insufficient supply of oxygen and glucose, cells primarily rely on anaerobic glycolysis to produce energy, with a significant reduction in ATP synthesis. Meanwhile, high-energy phosphate compounds such as creatine phosphate are gradually depleted. During reperfusion, although the supply of oxygen and glucose is restored, mitochondrial dysfunction limits the ability of cells to utilize oxygen, resulting in the slow recovery of aerobic metabolism and insufficient ATP synthesis. Additionally, during reperfusion, precursor substances for ATP synthesis, such as adenosine, inosine, and hypoxanthine, are washed out of the cells, depriving them of the material basis for resynthesizing high-energy phosphate compounds. This leads to the persistence of cellular energy metabolism disorders, which affect the normal functional recovery of cells.[26]

Leukocyte involvement

Experimental studies have shown a significant increase in leukocyte infiltration in the brain tissue during cerebral ischemia-reperfusion.[27] When tissues are damaged by ischemia, the degradation of cell membrane phospholipids and the increased metabolism of arachidonic acid produce substances with strong chemotactic effects, attracting a large number of leukocytes to enter the ischemic tissue and adhere to the endothelium. Leukocytes release various inflammatory mediators and chemokines, including leukotrienes, tumor necrosis factor-α (TNF-α), and interleukin-1 (IL-1), further promoting the aggregation and activation of leukocytes. Aggregated leukocytes can become lodged and block capillaries, thereby obstructing the blood flow. Leukocytes can also release lysosomal enzymes that directly damage the surrounding tissue cells. Furthermore, neutrophil-derived ROS generated during respiratory bursts significantly potentiates oxidative stress-mediated tissue injury.[28]

Comprehensive analysis of the NLRP3 inflammasome

Structural composition

NLRP3 inflammasome is a multiprotein complex composed of three central proteins: NLRP3 scaffold protein, caspase-1, and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). NLRP3 protein is part of the NOD-like receptor (NLR) family and is the main component of the NLRP3 inflammasome. The amino-terminal pyrin domain (PYD), central nucleotide-binding oligomerization domain (NACHT), and carboxyl-terminal leucine-rich repeat (LRR) domains.[29] The PYD domain primarily mediates the interaction between NLRP3 and ASC through PYD-PYD homotypic interactions. The NACHT domain, which hydrolyzes ATP, is crucial for NLRP3 activity. ATP binding and hydrolysis result in conformational changes in the NACHT domain, activating NLRP3. The LRR domain plays a major role in the recognition of different PAMPs and DAMPs, and is responsible for initiating the activation signal transduction of the NLRP3 inflammasome through binding.[7]

ASC is an adaptor protein that contains two domains: the amino-terminal PYD domain and carboxyl-terminal caspase recruitment domain

(CARD). ASC acts as a bridge in the NLRP3 inflammasome. NLRP3 forms a homotypic interaction through its PYD domain and a heterotypic interaction with pro-caspase-1 through CARD-CARD binding, thereby linking NLRP3 to pro-caspase-1 and enabling assembly and activation of the inflammasome.[30]

Caspase-1 serves as an effector protein in the NLRP3 inflammasome, and initially exists in an inactive pro-enzyme form (pro-caspase-1).[31] Pro-caspase-1 is recruited to the NLRP3 inflammasome complex via CARD-CARD interactions with ASC, after which it undergoes self-cleavage and activation influenced by the NLRP3 inflammasome. Mature caspase-1 exhibits potent substrate specificity and proteolytically processes pro-IL-1β and pro-IL-18 into their bioactive forms to initiate the inflammatory cascades. Additionally, caspase-1 cleaves gasdermin D (GSDMD), thereby releasing its N-terminal domain. The pore-forming activity of this domain induces pyroptotic cell death, a lytic programmed cell death pathway that amplifies inflammatory signaling.[32]

Function and activation mechanism

Basic activation process of the NLRP3 inflammasome

The NLRP3 inflammasome serves as a pivotal innate immune sensor that maintains metabolic homeostasis and coordinates host defense against pathogens by recognizing PAMPs, such as bacterial lipopolysaccharides (LPS), viral nucleic acids, and DAMPs, including ATP and uric acid crystals.[33] In bacterial infections, LPS binds to Toll-like receptor 4 (TLR4) on immune cells, activating NF-κB signaling to upregulate NLRP3 and pro-IL-1β transcription and prime the cells for inflammasome activation. NLRP3 oligomerizes with ASC and pro-caspase-1 to form a functional inflammasome complex. Activated caspase-1 cleaves pro-IL-1β and pro-IL-18 into mature cytokines, and recruits macrophages and neutrophils to clear pathogens. Additionally, caspase-1 processes gasdermin D (GSDMD), inducing pyroptosis, a lytic cell death mechanism that limits the spread of pathogens by eliminating the infected cells.[34]

In CIRI, aberrant NLRP3 activation exacerbates the pathology of neurons and glial cells (e.g. as microglia and astrocytes). DAMPs, such as ATP and high mobility group box 1 (HMGB1), released during ischemia, trigger NLRP3 activation.[35] In microglia, extracellular ATP activates P2X7 receptors, driving potassium efflux, which lowers intracellular K^+, a critical signal for NLRP3 assembly.[36] In neurons and astrocytes, mitochondrial dysfunction during reperfusion generates excessive reactive oxygen species (ROS), oxidizing mitochondrial DNA, which acts as a DAMP to amplify NLRP3 signaling.[37] This results in IL-1β and IL-18 release, intensifying neuroinflammation, neuronal apoptosis, and blood-brain barrier (BBB) disruption, which are core features of CIRI.[35,38] IL-1β upregulates endothelial adhesion molecules, promoting leukocyte infiltration, which exacerbates oxidative stress and endothelial damage, impairing microcirculation and worsening ischemic injury.[39] These processes link NLRP3 activation to CIRI pathologies such as lipid peroxidation and BBB breakdown.[40]

NLRP3 activation requires two signaling pathways. First, the priming signal involves TLR-mediated NF-κB activation, which enhances the expression of NLRP3 and pro-IL-1β.[41] The second signal triggers inflammasome assembly via pathways, such as potassium efflux, mitochondrial ROS production, and lysosomal damage. In CIRI, potassium efflux in neurons and microglia, driven by ionic imbalances, is a key activator.[36] Mitochondrial ROS, which are prevalent in ischemic neurons, connect oxidative stress to NLRP3 activation, whereas lysosomal damage in microglia releases cathepsin B, which further promotes NLRP3 assembly.[42] These mechanisms amplify CIRI's oxidative and endothelial damage caused by CIRI, contributing to neuronal loss and BBB dysfunction.[40]

In addition to CIRI, dysregulated NLRP3 activation drives pathology in Alzheimer's disease, Parkinson's disease, and type 2 diabetes, in which IL-1β release in adipose tissue macrophages impairs insulin sensitivity.[42] In atherosclerosis and autoimmune disorders, such as rheumatoid arthritis, NLRP3 exacerbates inflammation.[43,44] Elucidating NLRP3 activation in specific neural and glial cells and its role in oxidative and endothelial pathology of CIRI is essential for developing targeted therapies to mitigate neuroinflammation and improve neurological outcomes.[35]

It is worth noting that most of the current evidence for NLRP3 activation mechanisms in CIRI comes from middle cerebral artery occlusion (MCAO) mouse models, which have inherent limitations. For instance, the

pathological process of CIRI in humans involves more complex vascular and neurological characteristics compared to rodent models, and the short follow-up time of most animal experiments (usually within 7-14 days) fails to reflect the long-term effects of NLRP3 activation on neurological recovery.[45-47] Additionally, the reproducibility of some studies is limited by differences in animal strains, surgical procedures, and intervention timings[48], which should be considered when interpreting the results.

Key regulators of NLRP3 activation in CIRI

Several conserved signaling pathways and cellular processes critically regulate NLRP3 inflammasome activation in CIRI. Potassium efflux, induced by extracellular ATP binding to P2X7 receptors on microglia and neurons, reduces intracellular K^+ concentration to promote NLRP3 oligomerization and ASC interaction, a prerequisite for inflammasome assembly.[36] Mitochondrial ROS (mtROS) generated by reperfusion-induced mitochondrial dysfunction oxidizes mtDNA into DAMPs, which bind to NLRP3 to amplify its activation.[37] Additionally, NF-κB signaling, activated by TLRs upon DAMP recognition, upregulates NLRP3 and pro-IL-1β transcription to complete the priming step[41,49], while the ERK, JNK, and p38 MAPK pathways further enhance NLRP3 activation at transcriptional and post-translational levels.[50] Autophagy acts as a negative regulator of NLRP3 inflammasome in CIRI by mediating the lysosomal degradation of NLRP3, ASC, and caspase-1.[51] These regulators interact dynamically: NF-κB and MAPK pathways promote NLRP3 activation, while mtROS and potassium efflux serve as direct activation signals, and autophagy balances this process to prevent excessive inflammation. Their dysregulation in CIRI leads to exaggerated NLRP3 activation and subsequent neuroinflammatory damage.

Regulatory mechanisms

NLRP3 inflammasome is tightly regulated through transcriptional, post-translational, and intracellular signaling mechanisms and has significant implications in cerebral ischemia-reperfusion injury (CIRI). These regulatory pathways, particularly NF-κB, MAPK, and autophagy, interact dynamically to modulate NLRP3 activity, thereby influencing neuroinflammation and CIRI pathology.

Transcriptional regulation

NLRP3 transcription is orchestrated by key transcription factors, notably NF-κB, which is a master regulator of inflammation.[49] In CIRI, DAMPs, such as ATP, activate toll-like receptors (TLRs) in microglia and astrocytes, triggering NF-κB translocation to the nucleus. NF-κB binds to the NLRP3 gene promoter and upregulates NLRP3 and pro-IL-1β expression, thereby priming cells for inflammasome activation. In macrophages, lipopolysaccharide (LPS) enhances NLRP3 transcription via NF-κB, a process amplified in CIRI due to ischemic stress.[49] Interferon regulatory factors (IRF3/IRF7) also regulate NLRP3 transcription. In viral infections or CIRI, IRF3 activation upregulates NLRP3, thereby promoting inflammatory cascades in the neurons and glial cells. In CIRI, NF-κB-driven NLRP3 upregulation exacerbates neuroinflammation by increasing IL-1β levels, which disrupts the BBB and amplifies oxidative stress.[39]

Post-translational modification

Inflammasome activity is significantly controlled by post-translational modifications such as phosphorylation and ubiquitination. E3 ligases, such as ITCH and TRIM25, facilitate ubiquitin-mediated activation of NLRP3.[52] During CIRI, microglial ITCH polyubiquitinates NLRP3 via the K63-link, leading to downstream assembly of the NLRP3 inflammasome, resulting in augmented release of IL-1β and neuroinflammation.[53] On the other hand, DUBs such as USP5 and USP14 target NLRP3 and function to trim the chains of ubiquitin, decreasing the level of NLRP3 and shutting down the activity of the inflammasome. USP5 deubiquitinates CIRI to reduce neuronal inflammation, oxidative stress, and apoptosis. NLRP3 function is also affected by its phosphorylation. NLRP3 is directly phosphorylated by protein kinase C (PKC) to facilitate its assembly in microglial inflammasomes, whereas NLRP3 activity is inhibited by phosphorylation through TBK1, which may limit inflammation in CIRI.[54] By controlling the release of cytokines and pyroptosis, the modifications integrate NLRP3 regulation into CIRI pathologies, such as oxidative stress and endothelial damage.

Intracellular signal transduction

The NLRP3 inflammasome is regulated by the mitogen-activated protein kinase (MAPK) signaling pathway, which has three branches. ERK, JNK, and p38 are activated during inflammation and independently regulate NLRP3 inflammasome gene expression and signal activation through the phosphorylation of relevant transcription factors and downstream signaling mechanisms. It has been shown that NLRP3 transcription, along with pro-IL-1β transcription and assembly of the complex, are enhanced during the stimulation of macrophages with LPS via the activation of the three branches of the ERK, JNK, and p38 MAPK pathways.[50] Autophagy, the process through which cellular homeostasis is regulated by the conserved pathway of lysosomal degradation, also helps in the modulation of NLRP3 inflammasome activity and in the regulation of NLRP3 inflammasome activation by degrading components of NLRP3 inflammasome proteins." During inflammatory stimulation, autophagy-related proteins are activated to form autophagosomes, which engulf and degrade NLRP3, ASC, and other inflammasome components, thereby reducing the inflammasome assembly and activation. Experimental evidence indicates that the pharmacological induction of autophagy in inflammatory models effectively suppresses NLRP3 inflammasome activation and attenuates the associated inflammatory pathology.[51]

CLOSE ASSOCIATION BETWEEN THE NLRP3 INFLAMMASOME AND CEREBRAL ISCHEMIA-REPERFUSION INJURY

Inducing neuroinflammatory responses

In CIRI, damaged neurons, microglia, and astrocytes release DAMPs, such as ATP and high-mobility group box 1 (HMGB1), initiating NLRP3 inflammasome activation.[39] HMGB1 binds to Toll-like receptor 4 (TLR4) on microglia, triggering MyD88-dependent NF-κB signaling, which upregulates NLRP3 and pro-IL-1β transcription, and primes inflammasome assembly.[39] In neurons and astrocytes, reperfusion-induced mitochondrial dysfunction elevates ROS, oxidizing mitochondrial DNA, which acts as a DAMP to amplify NLRP3 activation.[37] Activated caspase-1 cleaves pro-IL-1β and pro-IL-18 to release mature cytokines, which polarize microglia and astrocytes toward a proinflammatory phenotype and thereby enhance TNF-α and IL-6 secretions.[39] These cytokines create an inflammatory network, recruiting neutrophils and macrophages to the ischemic brain tissue, exacerbating neuronal apoptosis and BBB disruption.[39] IL-1β upregulates endothelial adhesion molecules (e.g., intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), enhancing neutrophil-endothelial interactions, transendothelial migration, and oxidative stress, which damages endothelial cells and worsens CIRI pathology.[39] However, the translational value of these findings is constrained by model-specific limitations. The microglial activation pattern and cytokine secretion profile in middle cerebral artery occlusion (MCAO) mouse models may not fully recapitulate those in human CIRI, as human ischemic stroke often involves comorbidities (e.g., hypertension, diabetes) that are rarely replicated in young, healthy laboratory animals.[55,56] Pharmacological NLRP3 inhibition suppresses IL-1β and IL-18, and attenuates neuroinflammation, neuronal damage, and BBB disruption, thereby improving CIRI outcomes.[35]

Inducing pyroptosis

Pyroptosis, a form of regulated necrosis, is critically driven by NLRP3 inflammasome activation in CIRI. Activation of caspase-1 cleaves pro-IL-1β, pro-IL-18, and gasdermin D (GSDMD), promoting cytokine maturation and initiating pyroptotic cell death.[7] GSDMD's N-terminal domain of GSDMD, which is normally inhibited by its C-terminal fragment, is liberated through caspase-1 cleavage and oligomerizes to form 10–14 nm pores on the plasma membrane.[57] Pore formation increases membrane permeability, allowing ion efflux and water influx, resulting in cell swelling, membrane rupture, and ultimately, pyroptosis.[7] Neurons, microglia, and astrocytes are particularly vulnerable to NLRP3-mediated pyroptosis in CIRI. Astrocytes, stimulated by inflammatory mediators such as HMGB1 also undergo pyroptosis, releasing IL-1β and IL-18, which amplify neuroinflammation through paracrine effects.[35] Experimental inhibition of caspase-1 or GSDMD in CIRI models has been shown to reduce pyroptosis, protect the brain tissue, mitigate inflammation, preserve endothelial integrity, and improve neurological outcomes.[58] These findings highlight the central role of NLRP3-mediated pyroptosis in CIRI

pathogenesis and suggest a need for multiple therapeutic interventions.

Despite these promising results, most in vivo studies use young adult mice, and the efficacy of targeting NLRP3-mediated pyroptosis in aged animals (which are more clinically relevant to human stroke patients) remains unclear. Moreover, the lack of standardized methods for detecting pyroptosis (e.g., inconsistent use of GSDMD cleavage products as biomarkers) makes it difficult to compare results across different studies, affecting the reliability of conclusions.

Disruption of the blood-brain barrier

The BBB serves as a critical neuroprotective barrier that maintains the homeostasis of the central nervous system (CNS) and consists of cerebrovascular endothelial cells, the basal lamina, pericytes, and astrocytic end-feet.[59] TNF-α can induce endothelial cells to produce nitric oxide (NO) and prostaglandins, which cause vasodilation and endothelial cell damage, collectively exacerbating blood-brain barrier dysfunction. IL-6 can activate related signaling pathways, affecting the expression and distribution of key tight junction components, including occludin and zona occludens-1 (ZO-1), resulting in structural disintegration of tight junctions and compromised blood-brain barrier integrity.[40] Moreover, NLRP3 inflammasome-mediated pyroptotic cell death contributes to blood-brain barrier disruption. Pyroptosis in brain microvascular endothelial cells disrupts membrane integrity, leading to the leakage of intracellular components and subsequent impairment of blood-brain barrier function. Disruption of the blood-brain barrier allows harmful substances from the bloodstream, such as bacteria, viruses, toxins, and inflammatory cells, to enter the brain tissue, triggering more severe inflammatory responses and neuronal damage. Studies have shown that the pharmacological inhibition of NLRP3 inflammasome activity in cerebral ischemia-reperfusion models attenuates neuroinflammation by suppressing pro-inflammatory cytokine release, preserving blood-brain barrier function, and mitigating cerebral edema along with inflammatory cell infiltration.[60]

Impact on angiogenesis and neurogenesis

Angiogenesis and neurogenesis are crucial for functional recovery following cerebral ischemia. However, abnormal activation of the NLRP3 inflammasome significantly inhibits these processes, thereby hindering the recovery of brain function. In terms of angiogenesis, inflammatory factors released after NLRP3 inflammasome activation interfere with the signaling pathways of vascular endothelial growth factor (VEGF), a key factor for angiogenesis. VEGF mediates its pro-angiogenic effects through receptor binding to endothelial cells, triggering downstream signaling cascades that stimulate cellular proliferation, directional migration, and tubular structure formation. However, pro-inflammatory cytokines, including IL-1β and TNF-α, downregulate VEGF expression and bioactivity, while simultaneously impairing endothelial cell responsiveness to VEGF.[61] In terms of neurogenesis, after cerebral ischemia, endogenous neural stem cells undergo activation, followed by proliferation and subsequent differentiation into both neuronal and glial lineages, facilitating neural tissue repair.[62] However, the inflammatory response triggered by NLRP3 inflammasome activation inhibits the proliferative capacity and differentiation potential of neural stem cells. Pro-inflammatory mediators disrupt cytokine-growth factor equilibrium in the neural stem cell niche, impairing neuronal differentiation and enhancing gliogenesis. IL-6 can inhibit the proliferation of neural stem cells and the differentiation of neurons by activating the STAT3 signaling pathway.[63] The inflammatory response also increases oxidative stress levels in the neural stem cell microenvironment, damaging the DNA and mitochondria of neural stem cells and affecting their normal function. Studies have shown that, in cerebral ischemia-reperfusion injury models, inhibiting the activation of the NLRP3 inflammasome can improve angiogenesis and neurogenesis and promote brain function recovery.[64]

INTERVENTION STRATEGIES AND FUTURE PROSPECTS FOR TREATMENT

Summary of current interventions

NLRP3 inflammasome inhibitors

Glibenclamide, a sulfonylurea drug initially used for diabetes management, has recently been shown to inhibit the NLRP3 inflammasome. Studies have shown that glibenclamide can interact with specific binding sites on the

NLRP3 protein, preventing its activation and thereby inhibiting the assembly and activation of the NLRP3 inflammasome.[65,66] In animal experiments, administration of glibenclamide to models of cerebral ischemia-reperfusion injury resulted in a significant reduction in cerebral infarct size and improvement in neurological deficits. This effect is attributed to the inhibition of NLRP3 inflammasome activation, which reduces the release of the inflammatory cytokines IL-1β and IL-18, alleviates neuroinflammation, and decreases neuronal apoptosis and necrosis (Figure 3). The schematic diagram systematically outlines the multi-target protective mechanism of glibenclamide in CIRI. However, the clinical translation of glibenclamide for CIRI faces notable challenges. Species differences exist in the structural domain of NLRP3 between rodents and humans, leading to variations in binding affinity and inhibitory efficacy of glibenclamide. Pharmacokinetically, glibenclamide has a short half-life (approximately 2-4 hours) in the central nervous system due to poor blood-brain barrier penetration and rapid efflux by ATP-binding cassette transporters[67,68] distribution, metabolism, and excretion of drugs. Understanding these contributions early in drug discovery allows for more accurate projection of the clinical pharmacokinetics. One method to assess the impact of transporters in vivo involves co-dosing specific inhibitors. The objective of the present study was to optimize the dose and route of administration of a P-glycoprotein (P-gp. Safety concerns are also prominent: glibenclamide's primary hypoglycemic effect may cause life-threatening hypoglycemia in stroke patients, especially those with impaired glucose metabolism or concurrent antidiabetic medications.[69]

MCC950 is a specific NLRP3 inhibitor that directly targets NLRP3 protein, blocking its interaction with ASC and pro-caspase-1, thus inhibiting the formation of the NLRP3 inflammasome. Research has indicated that

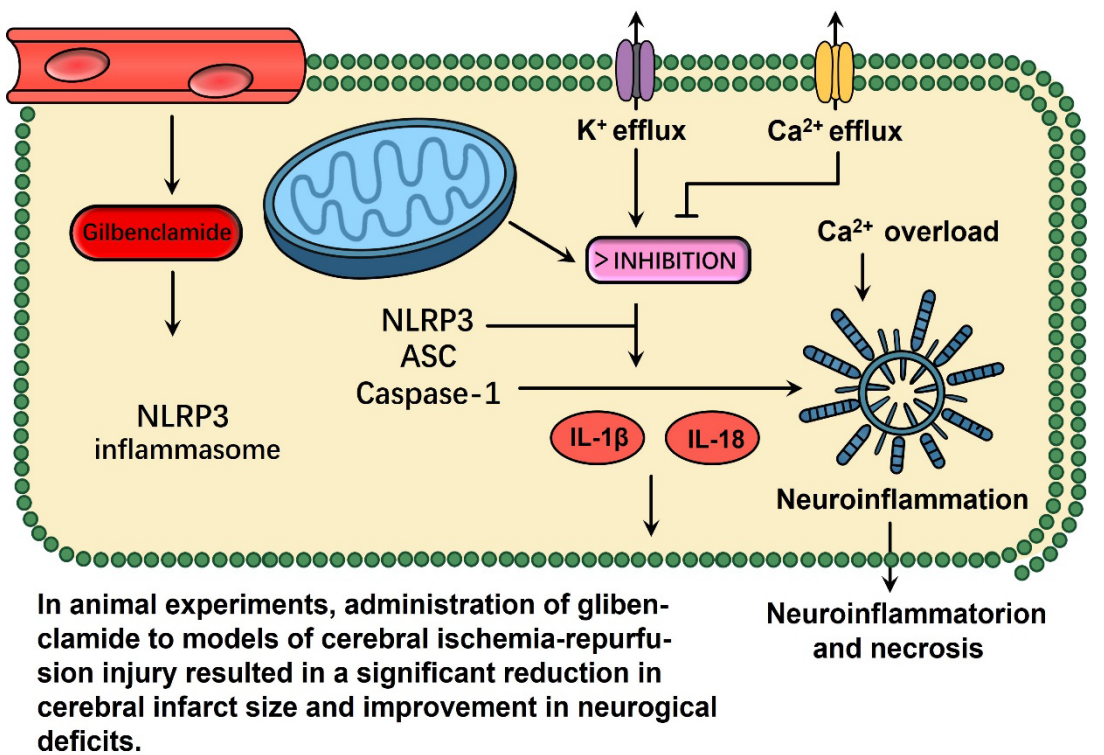

Figure 3. Schematic diagram of the protective effects of glibenclamide on cerebral ischemia–reperfusion injury: In animal models, administration of glibenclamide inhibits NLRP3 inflammasome activation by reducing mitochondrial dysfunction and ion flux disturbances (K^+ efflux, Ca^{2+} overload), thereby decreasing the expression of NLRP3, ASC, and caspase-1. This leads to reduced secretion of inflammatory cytokines IL-1β and IL-18, alleviating neuroinflammation, and decreasing neuronal apoptosis and necrosis, ultimately reducing cerebral infarct size and improving neurological outcomes.

MCC950 can significantly reduce NLRP3 inflammasome activation and IL-1β secretion induced by stimuli such as ATP and nigericin in cell experiments.[70] In animal models of cerebral ischemia-reperfusion injury, MCC950 markedly reduced inflammatory responses in the brain tissue, maintained the integrity of the blood-brain barrier, and improved neurological recovery compared with controls.[8,71] Notably, contradictory findings have emerged regarding the efficacy of MCC950 in CIRI. Several studies reported that MCC950 failed to exert significant neuroprotective effects in aged mouse CIRI models (≥18 months old), possibly due to age-related changes in NLRP3 protein structure or enhanced compensatory inflammatory pathways.[72–75] Additionally, some in vitro studies found that high concentrations of MCC950 may induce off-target effects (e.g., inhibition of other NLR family members)[76], which limits its clinical application potential. These inconsistent results highlight the need for further validation in more clinically relevant models.

Beyond inconsistent efficacy in aged models, MCC950 also encounters translational hurdles. Species-specific differences in NLRP3 protein stability and downstream signaling cascades result in reduced inhibitory activity in human cell lines compared to mouse models. Its poor aqueous solubility and low bioavailability (oral bioavailability <10%) limit clinical administration routes, while rapid hepatic metabolism further shortens its systemic exposure time. Although MCC950 shows higher specificity than glibenclamide, preclinical studies have reported mild hepatotoxicity at high doses, which requires rigorous safety assessment in clinical trials. Notably, no phase III clinical trials have been initiated for MCC950 in CIRI or other neurological disorders; current clinical data are limited to phase I/II trials for inflammatory diseases such as rheumatoid arthritis (ClinicalTrials.gov: NCT03878095), providing little direct evidence for its neurological application.

Traditional Chinese Medicine monomers

In addition to chemical synthetic inhibitors, natural products represented by traditional Chinese medicine (TCM) monomers have attracted increasing attention for their NLRP3 inflammasome inhibitory activity, due to their advantages of broad pharmacological effects and low toxic side effects. Several TCM monomers have been confirmed to alleviate CIRI by targeting the NLRP3 inflammasome. Baicalin, a flavonoid isolated from Scutellaria baicalensis, exhibits broad-spectrum pharmacological properties, including anti-inflammatory and antioxidant effects. In cerebral ischemia-reperfusion injury, baicalin has been shown to reduce neuroinflammatory responses by inhibiting the activation of the NLRP3 inflammasome.[77] Experimental evidence has demonstrated that baicalin can lower the expression levels of NLRP3, ASC, and caspase-1 in the brain tissue of animal models and reduce the release of IL-1β and IL-18. The therapeutic mechanism potentially involves suppression of the NF-κB pathway to attenuate NLRP3 transcription and expression, coupled with mitigation of mitochondrial impairment and ROS generation, consequently disrupting NLRP3 inflammasome activation signals. Additionally, baicalin can regulate autophagy, promote degradation of NLRP3 inflammasome-related proteins, and inhibit inflammasome activity.[78] However, current evidence has limitations: most studies lack positive controls and blind design, with inconsistent baicalin extraction methods affecting reproducibility. Dose-toxicity data are scarce, and its effects combine direct NLRP3 inhibition. All evidence is “in vivo/in vitro combined” without clinical validation.

Polygonum cuspidatum glycoside, one of the main active components of Polygonum cuspidatum, has recently been found to significantly inhibit the NLRP3 inflammasome. In models of cerebral ischemia-reperfusion injury, Polygonum cuspidatum glycoside can reduce brain tissue damage and improve neurological function. The underlying mechanism mainly involves blocking the formation of the NLRP3 inflammasome complex, thereby attenuating caspase-1 activation and subsequent IL-1β release.[79] Specifically, Polygonum cuspidatum glycoside can bind to specific domains of the NLRP3 protein, preventing the interaction between NLRP3 and ASC, thereby inhibiting inflammasome formation.[80] Moreover, it has antioxidant properties that can reduce oxidative stress-induced neuronal damage and indirectly alleviate neuroinflammation.[81] However, the main issues with these studies include a small sample size, non-standardization of the extracts (differences in plant sources/extract methods), and a narrow range of dose-response data.

Traditional Chinese Medicine formulas

On the basis of TCM monomers, classic TCM formulas with multi-component and multi-target characteristics show unique advantages in the treatment of CIRI, as they can regulate the NLRP3 inflammasome through multiple pathways and synergistically alleviate neuroinflammation and tissue damage.

Huangqi Guizhi Wuwu Tang 黄芪桂枝五物汤, a classic traditional Chinese medicine formula composed of Astragalus membranaceus, Cinnamomum cassia, Paeonia lactiflora, Zingiber officinale, and Jujuba dates, is known for its effects on invigorating qi, warming meridians, harmonizing blood, and alleviating arthralgia.[82]warming the meridians, and promoting blood circulation to alleviate obstruction. It is primarily used to treat conditions characterized by Qi stagnation, Yang deficiency, and obstruction, and it exhibits pharmacological effects such as immune regulation, anti-inflammation, analgesia, protection of the cardiovascular and cerebrovascular systems, itch relief, reduction of frostbite symptoms, antioxidative stress, promotion of cell apoptosis, and kidney protection. In modern clinical practice, it is commonly used to treat acute myocardial infarction, sequelae of cerebral infarction, cervical spondylosis, frozen shoulder, lower limb arteriosclerosis, lower limb vascular disorders, peripheral neuropathy in diabetes, and lupus nephritis. Recent research has focused on the chemical components, pharmacological effects, and clinical applications of Huangqi Guizhi Wuwu Decoction. Based on the " five principles" of quality markers(Q-markers This herbal formulation demonstrated cerebroprotective efficacy against cerebral ischemia-reperfusion injury through the suppression of NLRP3 inflammasome activation. Studies have demonstrated that Huangqi Guizhi Wuwu Tang treatment in cerebral ischemia-reperfusion injury models markedly downregulates NLRP3, caspase-1, and IL-1β expression in brain tissues, mitigates neuroinflammation, and reduces apoptotic neuronal cell death.[83]

Its mechanisms may involve the regulation of inflammatory signaling pathways, reduction of oxidative stress, and improvement of microcirculation.[84] Astragalus membranaceus in the formula can enhance the immune function of the body, regulate immune cell activity, and inhibit both inflammatory cell activation and cytokine secretion.[85] Cinnamomum cassia has the effect of warming and unblocking the meridians, improving cerebral blood circulation, and increasing the blood supply to ischemic brain tissue.[86] Paeonia lactiflora, Zingiber officinale, and Jujuba dates have harmonizing and antioxidant effects, collectively contributing to the alleviation of cerebral ischemia-reperfusion injury.[87,88]

The Yiqi Shengqing Formula，益气升清方 another traditional Chinese medicine formula based on the principles of TCM, is composed of Astragalus membranaceus, Codonopsis pilosula, Atractylodes macrocephala, Poria cocos, and Pueraria lobata, and has the ability to invigorate qi, clear the senses, and strengthen the spleen to resolve phlegm. Studies on cerebral ischemia-reperfusion injury have found that this formula can inhibit activation of the NLRP3 inflammasome, reduce neuroinflammatory responses, and improve neurological function. The experimental data revealed that the Yiqi Shengqing Formula downregulated the expression of NLRP3, ASC, and caspase-1 proteins in the cerebral tissues of animal models, while suppressing the release of the pro-inflammatory cytokines IL-1β and IL-18. Therapeutic mechanisms may include immunomodulatory effects, suppression of inflammatory cell recruitment and activation, and enhancement of cerebral energy metabolism to alleviate oxidative damage, consequently preventing NLRP3 inflammasome activation (Figure 4). This figure vividly demonstrates the multi-target regulatory effect of Yiqi Shengqing Formula on NLRP3 inflammasome activation in CIRI. The formula not only directly inhibits the assembly of NLRP3-ASC-caspase-1 complex but also indirectly suppresses NLRP3 activation by reducing mitochondrial ROS production, regulating ion efflux (K^+, Cl^-), and alleviating Ca^{2+} overload. The schematic also highlights the synergistic effects of its components: Astragalus membranaceus and Codonopsis pilosula enhance cerebral energy metabolism to reduce oxidative damage, while Pueraria lobata directly targets NLRP3.

Future research directions

Development of novel drugs

Although current NLRP3 inflammasome-targeted interventions have shown promising neuroprotective effects in preclinical CIRI models, there are still many translational

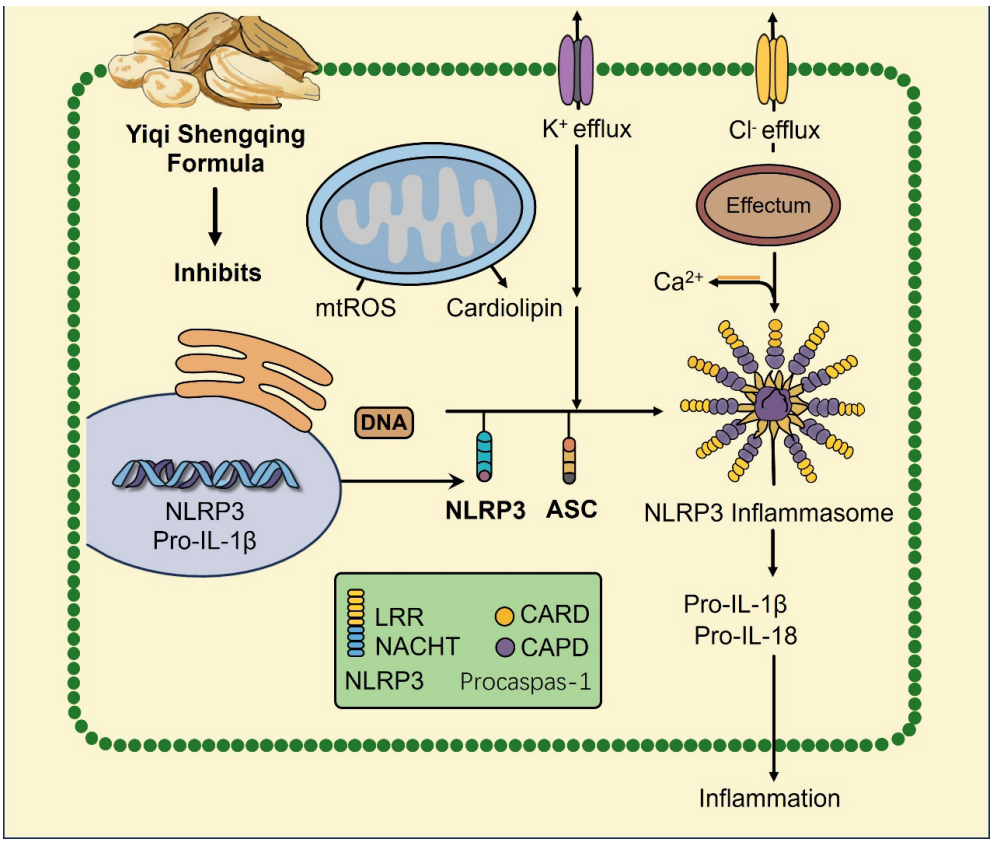

Figure 4. Schematic diagram of the mechanism of action: Yiqi Shengqing Formula, composed of Astragalus membranaceus, Codonopsis pilosula, Atractylodes macrocephala, Poria cocos, and Pueraria lobata, inhibits mtROS production, regulates ion efflux (K^+, Cl^-) and Ca^{2+} overload, blocks the assembly and activation of the NLRP3 inflammasome, reduces the protein expression of NLRP3, ASC, and caspase-1, and decreases the secretion of IL-1β and IL-18, thereby alleviating neuroinflammatory responses and improving neurological function after cerebral ischemia–reperfusion injury.

challenges limiting their clinical application, such as species differences, poor blood-brain barrier penetration and lack of standardization of TCM preparations. Addressing the translational gaps of current NLRP3 inhibitors is critical for advancing CIRI therapy. The primary obstacles include species differences in NLRP3 biology, inadequate blood-brain barrier penetration, safety risks, and lack of clinical validation. To overcome these, future drug development should prioritize: in-depth research on the structure and function of the NLRP3 inflammasome, using techniques such as structural biology and computer-aided drug design, can lead to the development of more specific, efficient, and less toxic NLRP3 inhibitors. For instance, by elucidating the three-dimensional structure of NLRP3 protein and identifying the key binding sites for ligands, small-molecule compounds that can precisely target these sites can be designed to enhance drug efficacy and safety.[89] Exploration of natural products with potential NLRP3 inhibitory activity from a wide range of plant, animal, and microbial sources can help identify novel active substances. Numerous phytochemicals have demonstrated polypharmacological effects with minimal toxicity and are capable of modulating NLRP3 inflammasome signaling through multiple pathways, thereby providing a rich resource for innovative drug development. Research on drugs targeting other key nodes in the NLRP3 inflammasome activation signaling pathway, in addition to directly inhibiting the NLRP3 protein, can also focus on upstream signaling molecules, transcription factors, or enzymes related to post-translational modifications in the inflammasome activation process.[90] Developing

multi-target combination therapies can improve treatment outcomes.

Exploration of combination therapy schemes

Combining NLRP3 inflammasome inhibitors with existing treatments for cerebral ischemia-reperfusion injury, such as thrombolytic therapy, neuroprotective drug therapy, and rehabilitation therapy, can help investigate the efficacy and safety of combination treatment regimens. For example, early administration of NLRP3 inhibitors in conjunction with thrombolytic therapy can be used to determine whether it can reduce reperfusion injury following thrombolysis and enhance neurological recovery. Exploring the combined use of NLRP3 inflammasome inhibitors with different mechanisms of action, as well as their combination with other anti-inflammatory drugs and antioxidants, can enhance the inhibitory effects on NLRP3 inflammasome through synergistic actions, thereby reducing inflammatory and oxidative stress damage. Clinical studies to validate the effectiveness and feasibility of combination therapy in patients with cerebral ischemia-reperfusion injury may provide additional options and evidence for clinical treatment. Large-scale multicenter clinical trials can assess the impact of combination therapy on patients' neurological recovery, quality of life improvement, and long-term prognosis, thereby promoting the clinical application of combination therapy.

Personalized treatment strategy research

Personalized treatment strategies for the NLRP3 inflammasome in each patient would require consideration of the patient's age, sex, comorbidities, and even their family tree. What remains to be seen is the extent to which these elements determine individual responses to NLRP3 inhibitors, and consequently, how these elements can be best utilized for fine-tuning therapy. Specific genetic polymorphisms may determine the rate of drug metabolism and the response to different classes of NLRP3 inhibitors, potentially leading to the need for changes in the drug dose or the drug itself. The more precisely the patient is characterized in terms of drug effectiveness and toxicity, the more optimally the patient can be treated.

Another important method involves integration of biomarkers into specific treatment strategies. These biomarkers can be used to predict individual responses to specific NLRP3-targeted therapies and to identify patients who would benefit the most from such treatments. Biomarkers can also help assess the degree of cerebral ischemia/reperfusion injury and monitor peripheral NLRP3 inflammasome activation, thereby providing data that can be used for real-time adaptive clinical decision making. Moreover, clinicians can accurately correlate therapeutic plans with specific treatment outcomes to optimize the management of cerebral ischemia-reperfusion injury. Finally, NLRP3 inflammasome strategies guided by personalized genetic and biomarker data stand to gain the most from the integration of these elements, minimizing the negative consequences of the prevalent generic approaches to therapy and enhancing the precision of care provided to patients.

CONCLUSION

This study systematically reviewed the inflammatory pathways in cerebral ischemia-reperfusion injury. As the core pathogenic hub of CIRI, the NLRP3 inflammasome integrates dual activation signals (NF-κB-mediated transcriptional initiation and potassium efflux/mtroS-driven post-translational activation) to exacerbate neuroinflammation, pyroptosis, blood-brain barrier destruction, and impaired neurogenesis/angiogenesis. Cell-specific activation of NLRP3 (microglia, neurons, astrocytes) leads to different CIRI pathologies, while its regulatory network (including the pre-activated NF-κB/MAPK pathway and inhibited autophagy) maintains a dynamic balance. Preclinical evidence consistently confirms NLRP3 as a viable therapeutic target, with inhibitors, TCM monomers, and formulations reducing IL-1β/IL-18 secretion and improving neurological outcome.

Notwithstanding these advances, critical knowledge gaps persist. The temporal-spatial dynamics of NLRP3 activation in CIRI, cell-specific regulatory mechanisms, and long-term effects of NLRP3 inhibition remain undefined. Contradictory efficacy in aged animal models and unclear crosstalk with other inflammatory pathways further hinder clinical translation. Additionally, TCM preparations lack standardization, and current NLRP3 inhibitors face species differences, pharmacokinetic challenges, and insufficient clinical validation.

Future research should prioritize cell-

specific NLRP3 inhibitors and standardized TCM formulations to enhance specificity and translatability. Combining NLRP3-targeted therapies with thrombolytics or antioxidants may synergistically mitigate CIRI, while validating NLRP3-related biomarkers will enable personalized treatment. Long-term preclinical studies and large-scale multicenter trials are essential to address age-related efficacy variations and safety concerns, ultimately bridging the gap between bench research and clinical application to reduce stroke-related disability and death.

DISCLOSURE

Financial support: This work was supported by the Natural Science Foundation of Hubei Province, China (Grant No. 2024 AFD199), and the Open Research Fund of the Mental Health Graduate Program at China Three Gorges University (Grant No. YCXL-23-19).

Conflict of interest: None

REFERENCES

1. Feigin V L, Brainin M, Norrving B, *et al.* World Stroke Organization: Global stroke fact sheet 2025. *Int J Stroke* 2025, 20(2): 132-44. doi: 10.1177/17474930241308142.
2. Huang J, Chen L, Yao ZM, *et al.* The role of mitochondrial dynamics in cerebral ischemia-reperfusion injury. *Biomed Pharmacother* 2023; 162: 114671. doi: 10.1016/j.biopha.2023.114671.
3. Awasthi V A, Dhankar V, Singh S. Novel therapeutic targets for reperfusion injury in ischemic stroke: Understanding the role of mitochondria, excitotoxicity and ferroptosis. *Vascul Pharmacol* 2024; 156: 107413. doi: 10.1016/j.vph.2024.107413.
4. Duan WL, Wang XJ, Ma YP, *et al*. Therapeutic strategies targeting the NLRP3-mediated inflammatory response and pyroptosis in cerebral ischemia/reperfusion injury (Review). *Mol Med Rep* 2024; 29(3): 46. doi: 10.3892/mmr.2024.13170.
5. Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. *Cell Mol Immunol* 2021; 18(9): 2114-27. doi: 10.1038/s41423-021-00740-6.
6. Franchi L, Eigenbrod T, Muñoz-Planillo R, *et al.* The inflammasome: A caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol* 2009; 10(3): 241-7. doi: 10.1038/ni.1703.
7. Kelley N, Jeltema D, Duan Y, *et al*. The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. *Int J Mol Sci* 2019; 20(13): 3328. doi: 10.3390/ijms20133328.
8. Franke M, Bieber M, Kraft P, *et al*. The NLRP3 inflammasome drives inflammation in ischemia/reperfusion injury after transient middle cerebral artery occlusion in mice. *Brain Behav Immun* 2021; 92: 223-33. doi: 10.1016/j.bbi.2020.12.009.
9. Cheng X, Yang YL, Li WH, *et al.* Cerebral ischemia-reperfusion aggravated cerebral infarction injury and possible differential genes identified by RNA-seq in rats. *Brain Res Bull* 2020; 156: 33-42. doi: 10.1016/j.brainresbull.2019.12.014.
10. Hou S, Zhang Y, Xia Y, *et al*. Global, regional, and national epidemiology of ischemic stroke from 1990 to 2021. *Eur J Neurol* 2024; 31(12): e16481. doi: 10.1111/ene.16481.
11. Lim L, Wang X, Yu Z. Ischemia-reperfusion injury in the brain: Mechanisms and potential therapeutic strategies. *Biochem Pharmacol* 2016; 5(4): 213. doi: 10.4172/2167-0501.1000213.
12. Zhang M, Liu Q, Meng H, *et al.* Ischemia-reperfusion injury: Molecular mechanisms and therapeutic targets. *Signal Transduct Targeted Ther* 2024; 9(1): 12. doi: 10.1038/s41392-023-01688-x.
13. Lee MH, Im SH, Jo KW, *et al.* Recanalization rate and clinical outcomes of intravenous tissue plasminogen activator administration for large vessel occlusion stroke patients. *J Korean Neurosurg Soc* 2023; 66(2): 144-54. doi: 10.3340/jkns.2022.0120.
14. Zhang Y, Tang H, Gui X, *et al.* Safety of recanalization therapy in acute ischemic stroke patients on direct oral anticoagulant therapy: An updated systematic review and meta-analysis. *Ann Indian Acad Neurol* 2022; 25(6): 1036-46. doi: 10.4103/aian.aian_271_22.
15. Zhang Y, Jiang M, Gao Y, *et al.* "No-reflow" phenomenon in acute ischemic stroke. *J Cereb Blood Flow Metab* 2024; 44(1): 19-37. doi: 10.1177/0271678X231208476.
16. Bekkers SCAM, Yazdani SK, Virmani R, *et al*. Microvascular obstruction: Underlying pathophysiology and clinical diagnosis. *J Am Coll Cardiol* 2010; 55(16): 1649-60. doi: 10.1016/j.jacc.2009.12.037.
17. Yue ZJ, Shi Z, Hu B, *et al*. [L-VDCC autoregulation abnormality contributes to calcium overload in myocardial ischemia-reperfusion injury] *Sheng Li Xue Bao* [*Acta Physiologica Sinica*] 2017; 69(6): 861-9.
18. Li YW, Liu Y, Luo SZ, *et al.* The significance of calcium ions in cerebral ischemia-reperfusion injury: Mechanisms and intervention strategies. *Front Mol Biosci* 2025; 12: 1585758. doi: 10.3389/fmolb.2025.1585758.
19. Ludhiadch A, Sharma R, Muriki A, *et al*. Role of calcium homeostasis in ischemic stroke: A review. *CNS Neurol Disord Drug Targets* 2022; 21(1): 52-61. doi: 10.2174/1871527320666210212141232.
20. Akhtar W, Muazzam Khan M, Kumar S, *et al.* Pathophysiology of cerebral ischemia-reperfusion injury: An overview of oxidative stress and plant-based therapeutic approaches. *Brain Res* 2025; 1847: 149308. doi: 10.1016/j.brainres.2024.149308.
21. Zhou T, Prather ER, Garrison DE, *et al.* Interplay between ROS and antioxidants during ischemia-reperfusion injuries in cardiac and skeletal muscle. *Int J Mol Sci* 2018; 19(2): 417. doi: 10.3390/ijms19020417.
22. Tang SP, Mao XL, Chen YH, *et al*. Reactive oxygen species induce fatty liver and ischemia-reperfusion

injury by promoting inflammation and cell death. *Front Immunol* 2022; 13: 870239. doi: 10.3389/fimmu.2022.870239.
23. Hemnani T, Parihar M S. Reactive oxygen species and oxidative DNA damage. *Indian J Physiol Pharmacol* 1998; 42(4): 440-52.
24. Feng S, Yang M, Liu S, *et al*. Oxidative stress as a bridge between age and stroke: A narrative review. *J Intensive Med* 2023; 3(4): 313-9. doi: 10.1016/j.jointm.2023.02.002.
25. Chen H, Yoshioka H, Kim GS, *et al.* Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. *Antioxid Redox Signal* 2011; 14(8): 1505-17. doi: 10.1089/ars.2010.3576.
26. Lei W, Zhuang H, Huang W, *et al*. Neuroinflammation and energy metabolism: A dual perspective on ischemic stroke. *J Transl Med* 2025; 23(1): 413. doi: 10.1186/s12967-025-06440-3.
27. Sienel RI, Kataoka H, Kim SW, *et al.* Adhesion of leukocytes to cerebral venules precedes neuronal cell death and is sufficient to trigger tissue damage after cerebral ischemia. *Front Neurol* 2021; 12: 807658. doi: 10.3389/fneur.2021.807658.
28. Mittal M, Siddiqui MR, Tran K, *et al*. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014; 20(7): 1126-67. doi: 10.1089/ars.2012.5149.
29. Franchi L, Warner N, Viani K, *et al.* Function of nod-like receptors in microbial recognition and host defense. *Immunol Rev* 2009; 227(1): 106-28. doi: 10.1111/j.1600-065X.2008.00734.x.
30. Xiao L, Magupalli VG, Wu H. Cryo-EM structures of the active NLRP3 inflammasome disc. *Nature* 2023; 613(7944): 595-600. doi: 10.1111/j.1600-065X.2008.00734.x.
31. Yu HB, Finlay BB. The caspase-1 inflammasome: A pilot of innate immune responses. *Cell Host Microbe* 2008; 4(3): 198-208. doi: 10.1016/j.chom.2008.08.007.
32. Sokolovska A, Becker CE, Ip WKE, *et al*. Activation of caspase-1 by the NLRP3 inflammasome regulates the NADPH oxidase NOX2 to control phagosome function. *Nat Immunol* 2013; 14(6): 543-53. doi: 10.1038/ni.2595.
33. Feng S, Wierzbowski MC, Hrovat-Schaale K, *et al*. Mechanisms of NLRP3 activation and inhibition elucidated by functional analysis of disease-associated variants. *Nat Immunol* 2025; 26(3): 511-23. doi: 10.1038/s41590-025-02088-9.
34. Pizzuto M, Hurtado-Navarro L, Molina-Lopez C, *et al.* Ornithine lipid is a partial TLR4 agonist and NLRP3 activator. *Cell Rep* 2024; 43(10): 114788. doi: 10.1016/j.celrep.2024.114788.
35. Xu W, Huang Y, Zhou R. NLRP3 inflammasome in neuroinflammation and central nervous system diseases. *Cell Mol Immunol* 2025; 22(4): 341-55. doi: 10.1038/s41423-025-01275-w.
36. Xu Z, Chen ZM, Wu X, *et al*. Distinct molecular mechanisms underlying potassium efflux for NLRP3 inflammasome activation. *Front Immunol* 2020; 11: 609441. doi: 10.3389/fimmu.2020.609441.
37. Heid ME, Keyel PA, Kamga C, *et al.* Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation. *J Immunol* 2013; 191(10): 5230-8. doi: 10.4049/jimmunol.1301490.
38. Lee J, Cho W, Yu J W, *et al.* NLRP3 activation induces BBB disruption and neutrophil infiltration via CXCR2 signaling in the mouse brain. *J Neuroinflammation* 2025; 22(1): 139. doi: 10.1186/s12974-025-03468-6.
39. Ghafouri-Fard S, Shoorei H, Poornajaf Y, *et al*. NLRP3: Role in ischemia/reperfusion injuries. *Front Immunol* 2022; 13: 926895. doi: 10.3389/fimmu.2022.926895.
40. Yang C, Hawkins K E, Doré S, *et al*. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. *Am J Physiol Cell Physiol* 2019; 316(2): C135-C153. doi: 10.1152/ajpcell.00136.2018.
41. Chen MY, Ye XJ, He XH, *et al*. The signaling pathways regulating NLRP3 inflammasome activation. *Inflammation* 2021; 44(4): 1229-45. doi: 10.1007/s10753-021-01439-6.
42. Wu KKL, Cheung SWM, Cheng KKY. NLRP3 inflammasome activation in adipose tissues and its implications on metabolic diseases. *Int J Mol Sci* 2020; 21(11): 4184. doi: 10.3390/ijms21114184.
43. Wang Z, Zhang S, Xiao Y, *et al.* NLRP3 inflammasome and inflammatory diseases. *Oxid Med Cell Longev* 2020; 2020: 4063562. doi: 10.1155/2020/4063562.
44. Blevins HM, Xu Y, Biby S, *et al.* The NLRP3 inflammasome pathway: A review of mechanisms and inhibitors for the treatment of inflammatory diseases. *Front Aging Neurosci* 2022; 14: 879021. doi: 10.3389/fnagi.2022.879021.
45. Mosneag IE, Flaherty SM, Wykes RC, *et al*. Stroke and translational research - Review of experimental models with a focus on awake ischaemic induction and anaesthesia. *Neuroscience* 2024; 550: 89-101. doi: 10.1016/j.neuroscience.2023.11.034.
46. Foddis M, Blumenau S, Mueller S, *et al.* Ide copy number variant does not influence stroke severity in 2 C57BL/6J mouse models nor in humans: An exploratory study. *Stroke* 2025; 56(3): 725-36. doi: 10.1161/STROKEAHA.124.049575.
47. Garcia-Bonilla L, Shahanoor Z, Sciortino R, *et al*. Analysis of brain and blood single-cell transcriptomics in acute and subacute phases after experimental stroke. *Nat Immunol* 2024; 25(2): 357-70. doi: 10.1038/s41590-023-01711-x.
48. Ström JO, Ingberg E, Theodorsson A, *et al.* Method parameters' impact on mortality and variability in rat stroke experiments: A meta-analysis. *BMC Neurosci* 2013; 14: 41. doi: 10.1186/1471-2202-14-41.
49. Tschopp J, Schroder K. NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol* 2010; 10(3): 210-15. doi: 10.1038/nri2725.
50. Li D, Ren W, Jiang Z, *et al.* Regulation of the NLRP3 inflammasome and macrophage pyroptosis by the P38 MAPK signaling pathway in a mouse model of acute lung injury. *Mol Med Rep* 2018; 18(5): 4399-409. doi: 10.3892/mmr.2018.9427.
51. Paik S, Kim JK, Shin HJ, *et al.* Updated insights into the molecular networks for NLRP3 inflammasome activation. *Cell Mol Immunol* 2025; 22(6): 563-96.

doi: 10.1038/s41423-025-01284-9.
52. Tang S, Geng Y, Wang Y, *et al.* The roles of ubiquitination and deubiquitination of NLRP3 inflammasome in inflammation-related diseases: A review. *Biomol Biomed* 2024; 24(4): 708-21. doi: 10.17305/bb.2023.9997.
53. Perez JM, Chen Y, Xiao TS, *et al*. Phosphorylation of the E3 ubiquitin protein ligase ITCH diminishes binding to its cognate E2 ubiquitin ligase. *J Biol Chem* 2018; 293(3): 1100-5. doi: 10.1074/jbc.RA117.000408.
54. Tang T, Li P, Zhou X, *et al.* The E3 ubiquitin ligase TRIM65 negatively regulates inflammasome activation through promoting ubiquitination of NLRP3. *Front Immunol* 2021; 12: 741839. doi: 10.3389/fimmu.2021.741839.
55. Li Z, Li M, Fang Z, *et al*. Immunological mechanisms and therapeutic strategies in cerebral ischemia-reperfusion injury: From inflammatory response to neurorepair. *Int J Mol Sci* 2025; 26(17): 8336. doi: 10.3390/ijms26178336.
56. Fan YY, Hu WW, Nan F, *et al.* Postconditioning-induced neuroprotection, mechanisms and applications in cerebral ischemia. *Neurochem Int* 2017; 107: 43-56. doi: 10.1016/j.neuint.2017.01.006.
57. Chu X, Zhang T, Bukhari I, *et al.* Ubiquitination of gasdermin D N-Terminal domain directs its membrane translocation and pore formation during pyroptosis. *Cell Death Dis* 2025; 16(1): 181. doi: 10.1038/s41419-025-07475-6.
58. Li W, Shen N, Kong L, *et al.* STING mediates microglial pyroptosis via interaction with NLRP3 in cerebral ischaemic stroke. *Stroke Vasc Neurol* 2024; 9(2): 153-64. doi: 10.1136/svn-2023-002320.
59. Alahmari A. Blood-brain barrier overview: Structural and functional correlation. *Neural Plast* 2021; 2021: 6564585. doi: 10.1155/2021/6564585.
60. Jurcau A, Simion A. Neuroinflammation in cerebral ischemia and ischemia/reperfusion injuries: From pathophysiology to therapeutic strategies. *Int J Mol Sci* 2021; 23(1): 14. doi: 10.3390/ijms23010014.
61. Zhang Y, Zhao X, Guo C, *et al*. The circadian system is essential for the crosstalk of VEGF-notch-mediated endothelial angiogenesis in ischemic stroke. *Neurosci Bull* 2023; 39(9): 1375-95. doi: 10.1007/s12264-023-01042-9.
62. Lee RHC, Lee MHH, Wu CYC, *et al*. Cerebral ischemia and neuroregeneration. *Neural Regen Res* 2018; 13(3): 373-85. doi: 10.4103/1673-5374.228711.
63. West AJ, Tsui V, Stylli SS, *et al.* The role of interleukin-6-STAT3 signalling in glioblastoma. *Oncol Lett* 2018; 16(4): 4095-104. doi: 10.3892/ol.2018.9227.
64. [64] region of SOCS3 attenuates IL6-induced proliferation and astrocytic differentiation of neural stem cells via cross talk between signaling pathways. *CNS Neurosci Ther* 2023; 29(1): 168-80. doi: 10.1111/cns.13992.
65. Mancuso R, Citterio LA, Agostini S, *et al*. Glibenclamide-loaded nanoparticles reduce NLRP3 inflammasome activation and modulate miR-223-3p/miR-7-1-5p expression in THP-1 cells. *Pharmaceuticals* 2023; 16(11): 1590. doi: 10.3390/ph16111590.
66. Qiu X, Wang Q, Hou L, *et al*. Inhibition of NLRP3 inflammasome by glibenclamide attenuated dopaminergic neurodegeneration and motor deficits in paraquat and maneb-induced mouse Parkinson's disease model. *Toxicol Lett* 2021; 349: 1-11. doi: 10.1016/j.toxlet.2021.05.008.
67. Marie S, Comtat C, Caillé F, *et al.* 11C-glyburide PET imaging unveils the negligible brain penetration of glyburide in humans. *Neurology* 2019; 92(17): 813-4. doi: 10.1212/WNL.0000000000007378.
68. Rowbottom C, Pietrasiewicz A, Tuczewycz T, *et al*. Optimization of dose and route of administration of the P-glycoprotein inhibitor, valspodar (PSC-833) and the P-glycoprotein and breast cancer resistance protein dual-inhibitor, elacridar (GF120918) as dual infusion in rats. *Pharmacol Res Perspect* 2021; 9(2): e00740. doi: 10.1002/prp2.740.
69. Sheth KN, Albers GW, Saver JL, *et al.* Intravenous glibenclamide for cerebral oedema after large hemispheric stroke (CHARM): A phase 3, double-blind, placebo-controlled, randomised trial. *Lancet. Neurol* 2024; 23(12): 1205-13. doi: 10.1016/S1474-4422(24)00425-3.
70. Zhang X, Wang Z, Zheng Y, *et al.* Inhibitors of the NLRP3 inflammasome pathway as promising therapeutic candidates for inflammatory diseases (Review). *Int J Mol Med* 2023; 51(4): 35. doi: 10.3892/ijmm.2023.5238.
71. Jiao J, Zhao G, Wang Y, *et al.* MCC950, a selective inhibitor of NLRP3 inflammasome, reduces the inflammatory response and improves neurological outcomes in mice model of spinal cord injury. *Front Mol Biosci* 2020; 7: 37. doi: 10.3389/fmolb.2020.00037.
72. Xu C, Zhu R, Dai Q, *et al.* The role and mechanism of the NLRP3-IL-1β/IL-18 signaling axis in the progression of sepsis under an aging phenotype. *Life Sci* 2025; 378: 123812. doi: 10.1016/j.lfs.2025.123812.
73. Yang W, Si SC, Li J, *et al*. NLRP3 inhibitor alleviates glycemic variability-induced cognitive impairment in aged rats with type 2 diabetes mellitus. *Mol Cell Endocrinol* 2025; 595: 112406. doi: 10.1016/j.mce.2024.112406.
74. Naeem A, Prakash R, Kumari N, *et al*. MCC950 reduces autophagy and improves cognitive function by inhibiting NLRP3-dependent neuroinflammation in a rat model of Alzheimer's disease. *Brain Behav Immun* 2024; 116: 70-84. doi: 10.1016/j.bbi.2023.11.031.
75. Bonfante S, Netto MB, De Oliveira Jun AN, *et al.* Oxidative stress and mitochondrial dysfunction contributes to postoperative cognitive dysfunction in elderly rats dependent on NLRP3 activation. *Metab Brain Dis* 2024; 40(1): 1. doi: 10.1007/s11011-024-01425-5.
76. Kim W, Kim S, Woo H, *et al*. A potent NLRP3 inhibitor effective against both MCC950-sensitive and -resistant inflammation. *Cell Chem Biol* 2025; 32(9): 1125-39.e7. doi: 10.1016/j.chembiol.2025.08.006.
77. Si L, An Y, Zhou J, *et al*. Neuroprotective effects of baicalin and baicalein on the central nervous system and the underlying mechanisms. *Heliyon* 2025; 11(1):

e41002. doi: 10.1016/j.heliyon.2024.e41002.
78. Zhang Q, Guo S, Wang H. The protective role of baicalin in the regulation of NLRP3 inflammasome in different diseases. *Cell Biochem Biophysics* 2025; 83(2): 1387-97. doi: 10.1007/s12013-024-01597-y.
79. Huang Q, Xin X, Sun Q, *et al*. Plant-derived bioactive compounds regulate the NLRP3 inflammasome to treat NAFLD. *Front Pharmacol* 2022; 13: 896899. doi: 10.3389/fphar.2022.896899.
80. Zhang X, Wang Z, Li X, *et al*. Polydatin protects against atherosclerosis by activating autophagy and inhibiting pyroptosis mediated by the NLRP3 inflammasome. *J Ethnopharmacol* 2023; 309: 116304. doi: 10.1016/j.jep.2023.116304.
81. Zhang H, Li Y, Xun Y, *et al*. Polydatin protects neuronal cells from hydrogen peroxide damage by activating CREB/Ngb signaling. *Mol Med Rep* 2022; 25(1): 9. doi: 10.3892/mmr.2021.12525.
82. Lu GY, Wang JY, Gao Z, *et al.* [Research progress on Huangqi Guizhi Wuwu Decoction and predictive analysis of quality markers] *Zhongguo Zhong Yao Za Zhi* 2023; 48(20): 5438-49. doi: 10.19540/j.cnki.cjcmm.20230516.201.
83. Ou Z, Zhao M, Xu Y, *et al.* Huangqi Guizhi Wuwu decoction promotes M2 microglia polarization and synaptic plasticity via sirt1/NF-κB/NLRP3 pathway in MCAO rats. *Aging* 2023; 15(19): 10031-56. doi: 10.18632/aging.204989.
84. Liao W, Wang M, Wu Y, *et al.* The mechanisms of Huangqi Guizhi Wuwu decoction in treating ischaemic stroke based on network pharmacology and experiment verification. *Pharm Biol* 2023; 61(1): 1014-29. doi: 10.1080/13880209.2023.2230477.
85. Lim SM, Park HB, Jin JO. Polysaccharide from astragalus membranaceus promotes the activation of human peripheral blood and mouse spleen dendritic cells. *Chin J Nat Med* 2021; 19(1): 56-62. doi: 10.1016/S1875-5364(21)60006-7.
86. Luo C, Chen Q, Liu B, *et al*. The extracts of *Angelica sinensis* and *Cinnamomum cassia* from oriental medicinal foods regulate inflammatory and autophagic pathways against neural injury after ischemic stroke. *Oxid Med Cell Longev* 2021; 2021: 9663208. doi: 10.1155/2021/9663208.
87. Orellana-Urzúa S, Briones-Valdivieso C, Chichiarelli S, *et al*. Potential role of natural antioxidants in countering reperfusion injury in acute myocardial infarction and ischemic stroke. *Antioxidants* 2023; 12(9): 1760. doi: 10.3390/antiox12091760.
88. Wang A, Zhao W, Yan K, *et al*. Preclinical evidence of paeoniflorin effectiveness for the management of cerebral ischemia/reperfusion injury: A systematic review and meta-analysis. *Front Pharmacol* 2022; 13: 827770. doi: 10.3389/fphar.2022.827770.
89. Sharma M, De Alba E. Structure, activation and regulation of NLRP3 and AIM2 inflammasomes. *Int J Mol Sci* 2021; 22(2): 872. doi: 10.3390/ijms22020872.
90. Bagherniya M, Khedmatgozar H, Fakheran O, *et al.* Medicinal plants and bioactive natural products as inhibitors of NLRP3 inflammasome. *Phytother Res* 2021; 35(9): 4804-33. doi: 10.1002/ptr.7118.