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REVIEW

Research Progress on the Pyroptosis Mechanism and Related Active Ingredients of Natural Drugs in Spinal Cord Injury

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Abstract: Spinal cord injury (SCI) is a severe neurotraumatic condition for which effective therapeutic options remain limited, and advances in clinical pharmacological research have been slow. The diverse pathophysiological alterations that occur after SCI initiate cellular pyroptosis, which in turn exacerbates tissue damage, impedes neuronal functional recovery, and connects multiple pathological processes involved in SCI. In recent years, research on natural bioactive compounds has made substantial progress in the field of neurotrauma, including SCI, leading to the identification of several compounds capable of effectively modulating pyroptosis and promoting functional recovery. Therefore, a comprehensive synthesis of the mechanisms underlying pyroptosis during SCI pathophysiology, along with an overview of natural bioactive constituents with the potential to modulate SCI-related pyroptosis, may provide useful insights for future pharmacological studies, mechanistic investigations, and clinical management of SCI.

Keywords: spinal cord injury, pyroptosis, natural bioactive compounds, central nervous system, NLRP3

Introduction

Spinal cord injury (SCI) is a neurological disorder resulting from traumatic insults to the central nervous system (CNS), including impact, shear, and torsional forces, that cause neuronal death, disrupt conduction pathways, and impair motor and sensory functions below the injury level, potentially leading to paralysis or death.[1] Following the primary insult, neuronal loss and activation of inflammatory cells within the injured tissue initiate secondary pathological processes such as axonal degeneration, pro-inflammatory cytokine accumulation, and demyelination, which together prolong and exacerbate the impairment of spinal sensory, motor, and autonomic functions.[2] Because primary damage is irreversible, therapeutic strategies prioritize the mitigation of these secondary microenvironmental disturbances and the promotion of neuronal regeneration. However, the financial burden of SCI is substantial: first-year treatment costs, including acute management and care for complications such as pressure ulcers, pain, deep vein thrombosis, and SCI-associated immunodeficiency syndrome, often exceed US $1 million per patient.[3] The global prevalence of SCI is estimated to range from 223 to 755 cases per million population, while its incidence is estimated at 10.4 to 83 cases per million population.[4]

SCI initiates a cascade of interrelated pathological events, oxidative stress, inflammation, apoptosis, and pyroptosis, that amplify one another through extensive crosstalk. Among these, pyroptosis, an inflammatory form of programmed cell death, has emerged as a key driver of neuronal loss and secondary inflammation after SCI, making its targeted modulation a promising therapeutic strategy. In the injured spinal microenvironment, pattern-recognition receptors assemble the NOD-like receptor protein 3 (NLRP3) inflammasome, leading to autocatalytic activation of cysteine-aspartic acid protease-1 (Caspase-1).[5] Active caspase-1 then cleaves gasdermin D (GSDMD), releasing its N-terminal

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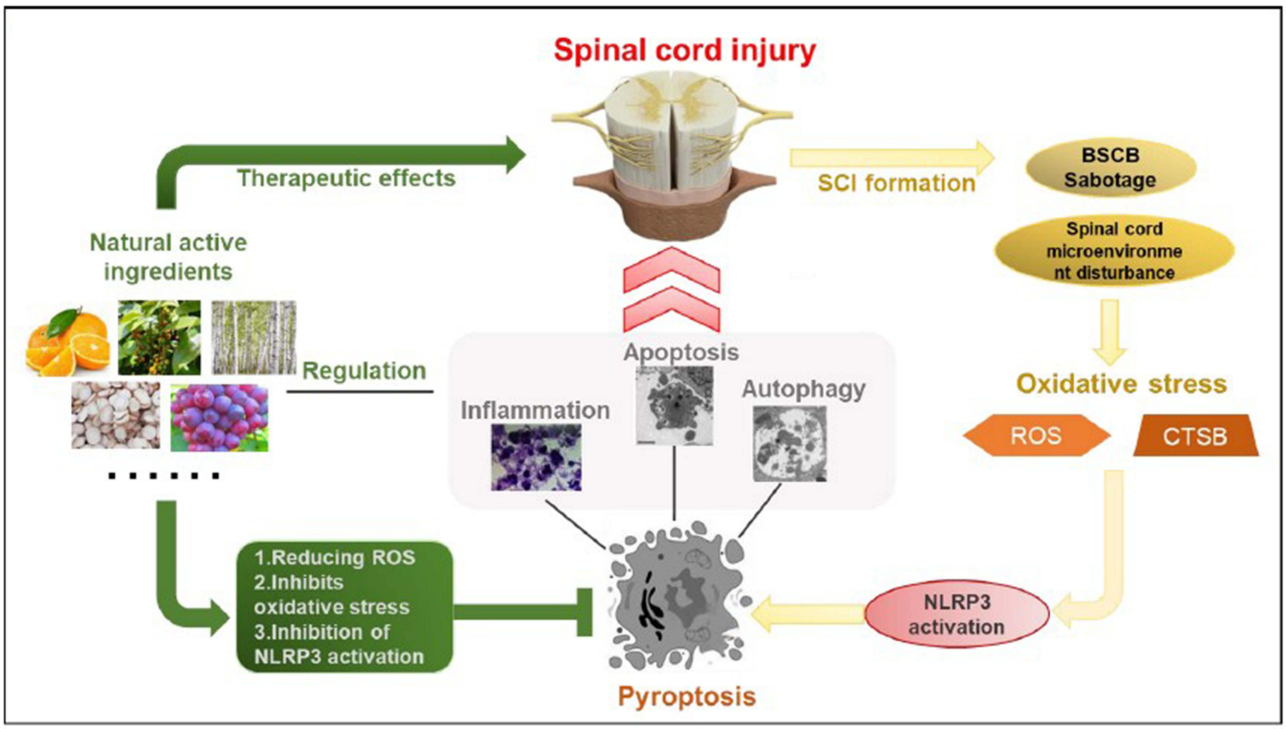
Graphical Abstract



fragment (GSDMD-N) to form pores in the cell membrane.[6] These pores permit efflux of water and pro-inflammatory cytokines, causing cell swelling, membrane rupture, and pyroptotic death, thereby exacerbating SCI-induced damage.[7]

Currently, no disease-modifying pharmacological therapy is available for patients with SCI. Rehabilitation remains the primary management strategy to improve functional outcomes and activities of daily living. Methylprednisolone is the only agent used clinically to mitigate inflammation associated with SCI, however, it is frequently accompanied by adverse events (AEs).[8,9] In addition, recent clinical studies have failed to demonstrate substantive improvements in tissue repair or motor function recovery in SCI patients.[10–12] Cell transplantation approaches largely remain at the preclinical or early clinical trial stage,[13] and their broad clinical application is hindered by limitations in trial design, ethical concerns surrounding human embryos, and risks of immune rejection.[14] In light of these limitations, there is increasing interest in natural bioactive compounds, which often demonstrate multifaceted efficacy in complex diseases. Here, we review the molecular mechanisms of pyroptosis in SCI and summarize current evidence for natural bioactive agents that modulate this pathway.

Pathophysiology of Spinal Cord Injury: From Primary Insult to Secondary Injury

Primary injury in SCI encompasses the immediate mechanical damage to the spinal cord, such as tearing, contusion, and compression, often accompanied by bone fragment impingement and rupture of spinal ligaments, which leads to vascular disruption and tissue infarction.[15] Nitric oxide (NO) is a key mediator of the inflammatory cascade following neural injury, and its synthesis and activity are regulated by nitric oxide synthases (NOS).[16] The NOS family comprises three isoforms: inducible, endothelial, and neuronal NOS. Moreover, inflammatory conditions characterized by the release of cytokines such as IL-1 are closely associated with upregulated expression of inducible NOS (iNOS) and increased NO production. These irreversible structural insults to neural parenchyma and plasma membranes precipitate a cascade of biochemical and biomechanical changes at the injury site, thereby triggering secondary injury processes.[17]

Secondary injury in SCI involves a complex interplay of vascular dysfunction, ionic imbalance, excitotoxicity, oxidative stress, inflammation, apoptosis, and pyroptosis.[17] Among these, early compromise of the blood–spinal cord barrier (BSCB) is a critical event that drives subsequent pathology. The BSCB normally isolates the CNS from the systemic circulation and controls molecular exchange between blood and spinal tissue.[18] Within minutes to hours after the primary mechanical insult, endothelial cells shed their glycocalyx, tight junctions become disrupted, the integrity of the BSCB is compromised, vascular permeability increases, and inflammatory responses and tissue edema ensue.[19,20] Between 3 and 7 days post-injury, microglia, neutrophils, and astrocytes become activated and release proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6, triggering an inflammatory storm, accompanied by progressive exacerbation of tissue edema.[21,22] By 7–14 days after injury, endothelial cells begin to re-express tight junction proteins and components of the basement membrane, initiating partial BSCB repair. During this stage, reactive astrocytes proliferate and form a glial scar,[23] which helps limit the spread of inflammation but concurrently impedes axonal regeneration. This increased permeability permits infiltration of blood components, inflammatory mediators, free radicals, and excitotoxins into the spinal cord, disrupting the microenvironment and accelerating axonal and oligodendrocyte damage. Consequently, early strategies to prevent BSCB breakdown and promote its restoration are essential to stabilize the CNS milieu and improve clinical outcomes.[22]

Glutamate is the principal excitatory neurotransmitter in the CNS, supporting neural development during embryogenesis but becoming excitotoxic after maturation.[24] After SCI, ischemia, hypoxia, and membrane disruption cause a rapid rise in extracellular glutamate.[25] Excess glutamate overactivates its receptors, triggering massive Ca^{2+} and Na^{+} influx and K^{+} efflux. Elevated intracellular Ca^{2+} activates mitochondrial NADPH oxidase (NOX), generating large amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS),[26] which impair mitochondrial function and induce oxidative stress. Major ROS include superoxide, hydroxyl radicals, hydrogen peroxide, and singlet oxygen.[27] Under physiological conditions, moderate ROS levels maintain redox balance and facilitate repair; in secondary injury, however, excessive ROS and RNS overwhelm defenses, damaging DNA and proteins and promoting cell death.[28] SCI-induced hypoxia further amplifies ROS production and mitochondrial protein oxidation, while concurrently activating nuclear factor kappa-B (NF-κB) signaling to increase pro-inflammatory cytokines (TNF-α, IL-1, IL-6), thereby exacerbating injury.[29] Moreover, Ca^{2+} overload can initiate intrinsic apoptotic pathways via caspases and calpains, degrading cytoskeletal and membrane proteins, triggering apoptosis, and compromising the BSCB.[30] Finally, dysfunction of Na^{+}/K^{+} ATPase leads to axolemmal depolarization, excessive Na^{+} influx, cellular swelling, and axonal acidosis.[26]

Within 24 hours of SCI, neutrophils are the first immune responders, migrating to the lesion site in response to chemokines, cytokines, and upregulated endothelial adhesion molecules.[31] Once at the injury site, they release inflammatory mediators and proteolytic enzymes that aggravate tissue edema and necrosis. In parallel, resident microglia become activated[32] and polarize into pro-inflammatory M1 and anti-inflammatory M2 phenotypes. M1 microglia secrete TNF-α, IL-6, IL-12, iNOS, hypochlorous acid, and ROS, thereby promoting neuronal death.[33] Additionally, infiltrating immune cells generate excessive ROS, triggering oxidative stress and activating the microglial NLRP3 inflammasome, which leads to maturation and release of IL-1β and IL-18, further amplifying neuroinflammation.[34]

Notably, NLRP3 inflammasome activation is the pivotal initiating event in pyroptosis, which functions as a central hub connecting diverse pathological processes after SCI. In addition, mitochondrial dysfunction caused by calcium overload and disturbances in ionic homeostasis can also precipitate pyroptotic signaling. These mechanisms will be explored in detail below. Figure 1 summarizes the key pathological alterations and primary processes underlying spinal cord microenvironment disruption following SCI.

The Role of Pyroptosis in the Pathophysiological Processes of Spinal Cord Injury

Pyroptosis: An Overview

Pyroptosis, first described by Brennan and Cookson in 2001, is a distinct form of programmed cell death characterized by progressive cell swelling, plasma membrane rupture, and the release of intracellular contents that provoke a robust inflammatory response.[35] Mechanistically, pyroptosis relies on activation of inflammatory caspases and the pore-forming

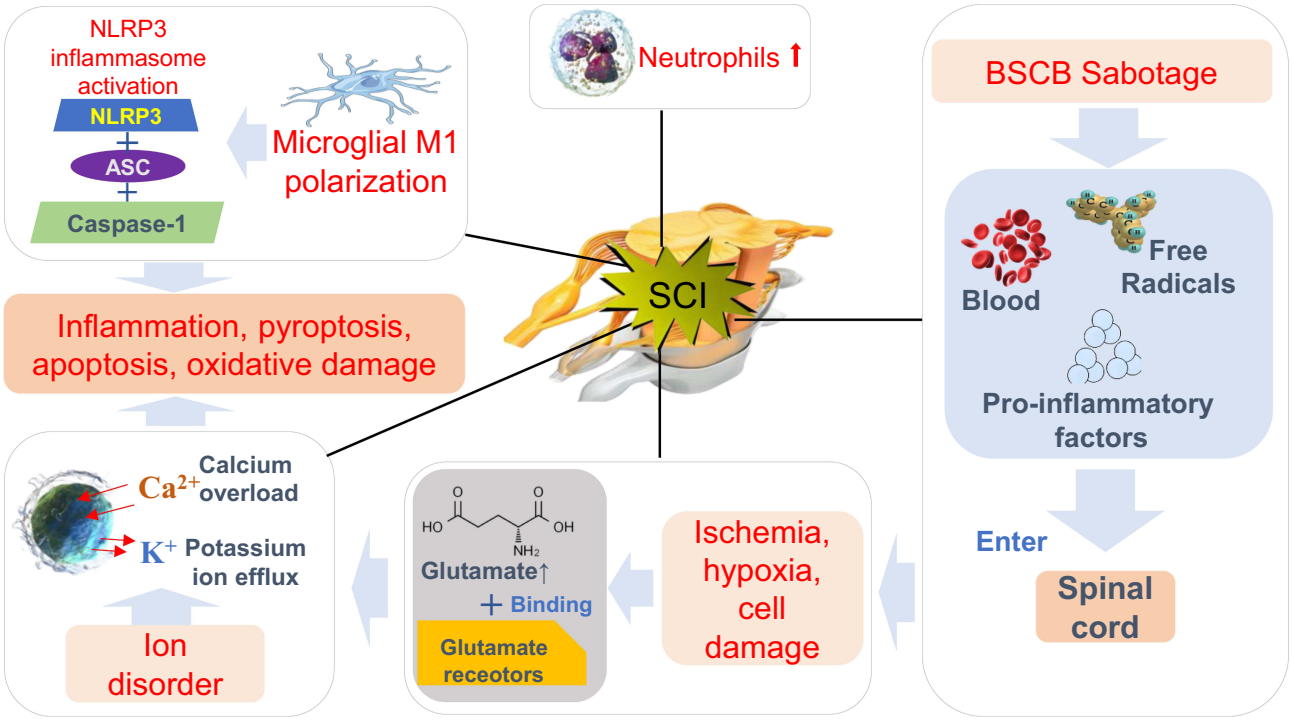


Figure 1 Disturbance of local microenvironment in spinal cord injury(SCI). Physical trauma induces SCI, resulting in breakdown of the blood-spinal cord barrier(BSCB), neutrophil infiltration, macrophage activation, increased glutamate excitotoxicity, and ion dysregulation, collectively disrupting the injury microenvironment and predisposing it to pyroptosis and further pathological sequelae. (Solid black line, SCI-related pathological processes/links; Light blue arrow, Indicates promotion of/causality in related processes; Red text, Key processes/factors).

Abbreviations: SCI, Spinal Cord Injury; BSCB, Blood-Spinal Cord Barrier; NLRP3, NOD-like receptor thermal protein domain associated protein 3; ASC, Apoptosis-associated speck-like protein containing a CARD; Caspase-1, Cysteine-aspartic acid protease-1.

activity of Gasdermin family proteins, whose oligomerization in the plasma membrane compromises membrane integrity and results in proinflammatory cell lysis.[36] As discussed above, activation of the NLRP3 inflammasome serves as a key upstream trigger of pyroptosis. To date, three principal pyroptotic pathways have been defined: the canonical pathway mediated by caspase-1 downstream of NLRP3, the non-canonical pathway driven by caspase-11 in mice (caspase-4/5 in humans), and alternative routes induced by various stimuli such as pharmacological agents.[37] The canonical pathway of NLRP3-mediated pyroptosis is primarily initiated when cells sense danger-associated signals, leading to assembly of the NLRP3 inflammasome and subsequent activation of caspase-1. Activated caspase-1 cleaves GSDMD, generating an N-terminal fragment that forms membrane pores, thereby inducing osmotic swelling and lytic cell death. Caspase-1 also processes proinflammatory cytokine precursors, releasing mature IL-1β and IL-18 to further drive inflammation.[38] For example, following SCI, various molecules can traverse the disrupted BSCB and enter the neuronal cytosol[39] and intracellular invasion by pathogens such as *Salmonella* or *Shigella* can similarly activate this pathway.[40] In contrast, the noncanonical pathway, mediated by caspase-4/5/11, operates independently of the canonical inflammasome.[41] Upon entering host cells, lipopolysaccharide (LPS) is directly recognized and bound by caspase-4/5 (in humans) or caspase-11 (in mice), triggering their activation. These caspases subsequently cleave GSDMD, inducing pyroptotic cell death.[42] Although this pathway does not initially produce IL-1β directly, it promotes K^+ efflux, which can secondarily activate the NLRP3 inflammasome.[43] Additional alternative pyroptotic pathways can be triggered by specific pharmacological agents (eg, cisplatin, doxorubicin, trametinib) or stimuli (eg, massive K^+ efflux, viral proteins), whereby caspase-3 becomes activated and cleaves another gasdermin family member, gasdermin E (GSDME),[44] thereby converting apoptotic signaling into a pyroptotic outcome. Moreover, granzymes and noncanonical inflammasomes such as absent in melanoma 2 (AIM2) and NOD-like receptor family CARD domain-containing protein 4 (NLRC4) can activate distinct caspases (eg, caspase-8) or directly engage gasdermins to induce pyroptosis.[45,46]

In SCI, the canonical NLRP3 pathway is particularly prominent: danger-associated molecular patterns (eg, extracellular ATP) engage pattern recognition receptors to promotes NLRP3 inflammasome assembly, leading to pro-caspase -1 activation, caspase-1–mediated cleavage of GSDMD into its N-terminal fragment, and processing of pro-IL-1β and pro-IL-18 into their mature forms.[47] The resulting GSDMD-N pores disrupt membrane permeability, collapse osmotic gradients, induce cell swelling and membrane rupture, release cytosolic contents, and culminate in cell death.[48]

Wang et al[49] reported that the mRNA levels of pyroptosis-related molecules, including NLRP3, GSDMD, apoptosis-associated speck-like protein containing a CARD (ASC), and caspase-1, were significantly upregulated in the spinal cord tissues of mice in the SCI group compared with controls. Consistently, enzyme linked immunosorbent assay (ELISA) analysis revealed elevated serum concentrations of IL-1β and IL-18 in SCI mice. Consistently, Xu et al[50] demonstrated that pharmacological attenuation of neuronal pyroptosis after SCI reduced IL-1β and IL-18 expression, mitigated neuronal damage, and improved functional neurological outcomes. These findings underscore that, following SCI, multiple CNS cell types, including neurons, microglia, and astrocytes, can undergo pyroptosis, thereby amplifying inflammation and tissue injury. Indeed, high expression of cytosolic inflammasome complexes, particularly NLRP3, has been observed in SCI and is known to drive microglial pyroptosis.[51] Moreover, Shan et al[52] revealed that dysregulation of the miR-325-3p/GSDMD axis in spinal cord tissue post-SCI induces astrocyte pyroptosis, further contributing to secondary injury.

Activation of NLRP3 and Onset of Pyroptosis in Spinal Cord Injury

Dysregulation of Potassium and Calcium Ions

Following SCI, ionic imbalances (particularly of K^+) are among the earliest and most significant pathological events. One of the first changes is K^+ efflux: Li et al[53] demonstrated that extracellular K^+ levels are significantly elevated in SCI rats compared to controls. Moreover, studies have reported that the potassium channel blocker 4-aminopyridine can effectively restore neural conduction in pediatric patients with SCI.[54] Furthermore, a meta-analysis indicated that 4-aminopyridine treatment can also improve motor and sensory function, sphincter control, and activities of daily living in patients with SCI.[55] These findings indicate that K^+ efflux is both widespread and central to SCI pathogenesis. Intracellular K^+ concentration also regulates inflammasome activation: elevated intracellular K^+ suppresses NLRP3 inflammasome assembly in response to diverse stimuli, whereas efflux-induced K^+ depletion facilitates its activation.[56] Conversely, NLRP3 inflammasome activation may further promote K^+ efflux, as Perregaux et al[57] observed that treating macrophages with NLRP3 agonists reduces intracellular K^+ and induces cellular swelling. Together, these data suggest that K^+ efflux, and the resulting intracellular K^+ depletion, are necessary prerequisites for NLRP3 inflammasome activation after SCI.[58]

Intracellular Ca^{2+} overload is a hallmark of SCI-induced ionic imbalance. Winkler et al[59] demonstrated that, within 30 minutes of injury, axonal Ca^{2+} begins to accumulate while extracellular Ca^{2+} levels rapidly decline; axonal Ca^{2+} peaks at 8 hours post-injury and remains elevated for up to a week. Two primary mechanisms link Ca^{2+} dysregulation to NLRP3 inflammasome activation. First, Ca^{2+} directly promotes spontaneous assembly of NLRP3 with its adaptor protein ASC, triggering inflammasome formation, although the precise Ca^{2+} binding sites and assembly process remain unclear. Second, excessive Ca^{2+} release from the endoplasmic reticulum induces mitochondrial damage and Ca^{2+} overload, leading to the release of mitochondrial DNA and reactive oxygen species that further amplify NLRP3 inflammasome activation.[60] Additionally, the calcium-sensing receptor (CaSR) enhances inflammasome activation by increasing intracellular Ca^{2+} and lowering cyclic adenosine monophosphate (cAMP) levels, conversely, CaSR knockdown attenuates NLRP3 inflammasome activation in response to canonical agonists.[61] Together, these findings underscore the multifaceted role of Ca^{2+} homeostasis in driving NLRP3-mediated inflammatory cell death after spinal cord injury.[60]

Oxidative Stress

Oxidative stress plays a pivotal role in the secondary injury cascade following SCI, and it is primarily driven by mitochondrial dysfunction. Mitochondria is the key regulators of processes such as pyroptosis and autophagy, generate most intracellular ROS via the electron transport chain.[62] Under physiological conditions, axonal Ca^{2+} buffering systems remove excess Ca^{2+} to maintain ionic homeostasis,[63] however, SCI-induced demyelination depletes ATP, impairs Ca^{2+}

buffering, and leads to intracellular Ca^{2+} overload.[64] Elevated Ca^{2+} levels activate mitochondrial complex I (NADH dehydrogenase), increasing electron leakage and ROS production.[65] Excess ROS then promote pore formation in the mitochondrial membrane, increasing its permeability and permitting further Ca^{2+} influx; this disrupts membrane potential and the respiratory chain.[66] The resulting mitochondrial permeability transition sustains a vicious cycle of chronic ROS release, mitochondrial damage, and activation of mitophagy pathways, which in turn generate additional ROS and perpetuate oxidative stress.

After CNS injury, infiltrating inflammatory cells become significant sources of ROS. In the acute phase of SCI, neutrophils rapidly migrate to the lesion site and release myeloperoxidase, elastase, and ROS.[67,68] Simultaneously, circulating monocytes infiltrate the spinal cord and differentiate into macrophages that phenotypically and functionally resemble activated microglia, together amplifying oxidative damage.[69] Resident microglia are also activated after SCI, polarize toward the proinflammatory M1 phenotype, and proliferate at the injury site. The NOX family of NADPH oxidases, including NOX1–5, dual oxidase 1(DUOX1), and DUOX2 serves as the primary enzymatic source of ROS in these myeloid cells. Among these, NOX2 is the predominant isoform in the CNS,[70] and its upregulation in microglia has been implicated in SCI, Parkinson's disease, and ischemic stroke. Gp91phox, the catalytic subunit of NOX2, is essential for this process: knockout of gp91phox in mice markedly reduces CNS oxidative stress.[71] Through NOX2-dependent mechanisms, activated microglia and infiltrating macrophages generate high levels of ROS,[72] as well as proteases and inflammatory mediators, thereby exacerbating the oxidative microenvironment of the injured spinal cord.[73]

Endoplasmic reticulum (ER) stress constitutes another major source of ROS that drives NLRP3 inflammasome activation through mitochondrial calcium overload. In the aftermath of SCI, inflammation and glutamate-mediated excitotoxicity impair ER function, inducing protein misfolding and disrupting ER Ca^{2+} homeostasis.[73] During oxidative protein folding, protein disulfide isomerase (PDI) catalyzes disulfide bond formation by accepting electrons from folding substrates,[74] these electrons are then transferred to ER oxidase 1α (ERO1α), which generates ROS. Concurrently, excessive Ca^{2+} release from the stressed ER at ER–mitochondrial contact sites precipitates mitochondrial dysfunction and further elevates mitochondrial ROS (mtROS), thereby amplifying NLRP3 inflammasome activation.

Numerous studies have confirmed that excessive ROS are essential for NLRP3 inflammasome activation. Cai et al reported that serum ROS levels in SCI rats were significantly higher than those in control rats, concomitant with elevated NLRP3 expression.[75] Other studies have shown that the saturated fatty acid palmitate can activate the NLRP3 inflammasome in bone marrow-derived mesenchymal stem cells and promote mitochondrial IL-1β release in a ROS-dependent manner,[76] Nakahira et al[77] demonstrated that mtROS generated by dysfunctional mitochondria in macrophages is a prerequisite for NLRP3 inflammasome activation under LPS stimulation, while cytosolic release of mitochondrial DNA also depends on both NLRP3 and mtROS. Furthermore, various NLRP3 inflammasome agonists can trigger mtROS production in multiple cell types, highlighting the critical role of ROS as a signaling mediator for NLRP3 inflammasome activation.[78] Conversely, inhibiting NLRP3 can attenuate ROS levels: A study demonstrated that the natural compound formulation Rongmu Fugan formula reduced NLRP3 expression in both liver tissues of mice with cholestatic liver injury and in normal human hepatocytes. Concurrently, it decreased cellular ROS levels, the expression of inflammatory mediators, and oxidative stress, suggesting that inhibition of NLRP3 inflammasome activation may negatively regulate ROS production.[79] As mentioned above, ROS production also activates the NF-κB signaling pathway. Studies using SCI rat models and mouse microglial (BV2) cell inflammation models have shown that following spinal cord injury, phosphorylation of NF-κB p65 translocates from the cytoplasm to the nucleus, initiating transcription of target genes and promoting NLRP3 inflammasome activation. This, in turn, leads to activation of caspase-1 and ASC, facilitating GSDMD cleavage and pyroptotic cell death.[80,81] Together, ROS-driven oligomerization of NLRP3 with ASC and pro-caspase-1, caspase-1 activation, GSDMD cleavage to GSDMD-N, and maturation/secretion of IL-1β and IL-18, culminate in pyroptosis and amplified inflammatory responses.

Release of Cathepsin B

Cathepsins are a family of lysosomal cysteine proteases comprising 11 isoforms in humans, among which cathepsin B (CTSB) plays a central role in degrading extracellular, plasma, hormonal, and microbial proteins to maintain intracellular protein homeostasis. When lysosomal integrity is compromised, such as after macrophages phagocytose

particulates like silica or monosodium urate crystals, CTSB is released into the cytosol, serving as a hallmark of lysosomal dysfunction and a critical trigger for NLRP3 inflammasome assembly.[76] In hyperuricemia models, co-culture of uric acid (UA) with normal human liver (L-O2) cells markedly upregulates both inflammatory cytokines and CTSB expression,[82] suggesting that elevated systemic UA in SCI patients may similarly exacerbate CTSB release. Indeed, Ellis et al[83] reported sustained CTSB elevation for up to one week following SCI, implicating CTSB in secondary injury. Xu et al[84] analyzed multiple transcriptomic datasets to identify differentially expressed genes and found that the *Ctsb* gene was significantly dysregulated. It was highly expressed in myeloid cells following SCI and was broadly distributed throughout the lesion core. Pharmacological inhibition of CTSB with CA-074 methyl ester (CA-074-Me) significantly attenuates NLRP3 inflammasome activation in macrophages,[85] and *Ctsb* knockdown via small interfering RNA (siRNA) reduces hydroxyapatite crystal–induced inflammasome activation and pyroptosis in chondrocytes.[86] Collectively, these findings indicate that cytosolic CTSB accumulation is a key upstream event in UA- and particulate-induced NLRP3-dependent pyroptotic cell death.

Recent studies indicate that after SCI, activation of cytosolic phospholipase A_2 (PLA_2) triggers lysosomal membrane permeabilization, resulting in the release of CTSB into the cytosol and subsequent NLRP3 inflammasome activation.[87] In vitro experiments by Liu et al[88] demonstrated that PLA_2 activation leads to neuronal death. Correspondingly, both pharmacological inhibition and genetic ablation of PLA_2 in vivo significantly attenuate post-SCI inflammation, decrease cell death and tissue damage, and enhance motor function recovery. These findings underscore a pivotal role for PLA_2 in SCI pathophysiology and implicate it as a key mediator of lysosomal CTSB release. Nevertheless, the exact cellular sources and mechanisms governing CTSB release following SCI remain incompletely defined; clarifying these origins and their link to pyroptotic signaling is essential for elucidating CTSB's mechanistic contributions.

Figure 2 summarizes the mechanisms underlying pyroptosis and the key pathological processes following SCI.Ionic disturbances (notably K^+ efflux and Ca^{2+} overload), excessive ROS generation, and CTSB release together drive NLRP3

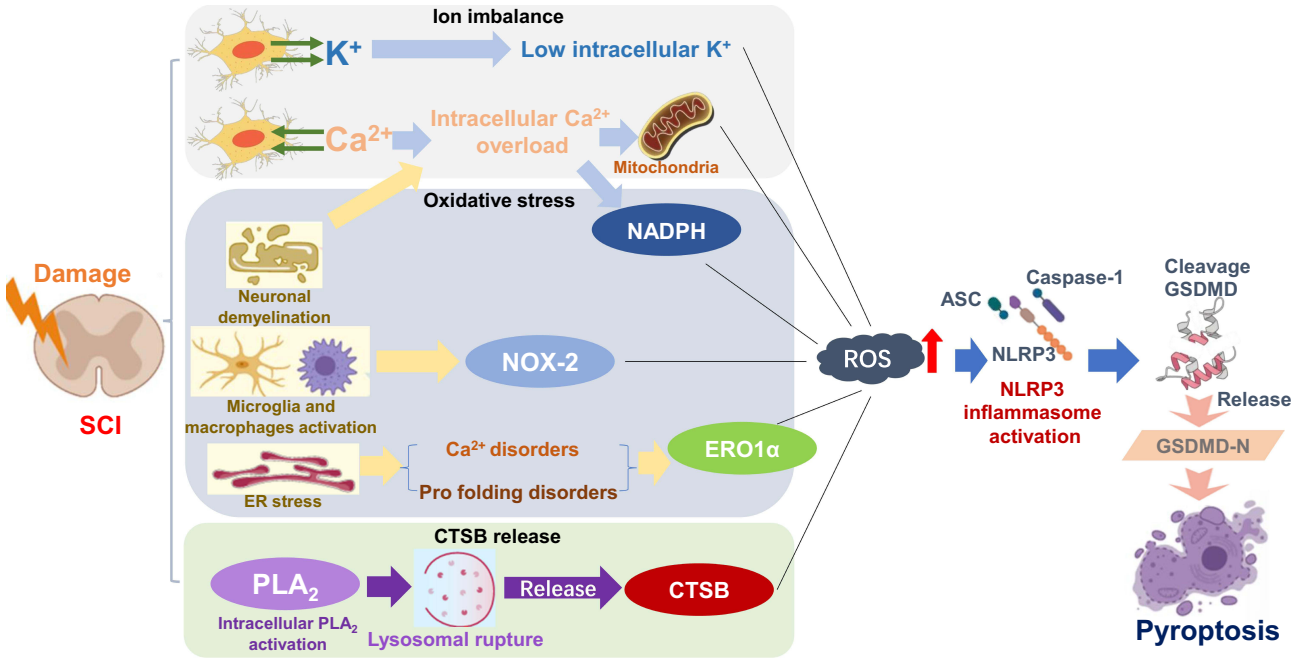


Figure 2 Initiation of pyroptosis in spinal cord injury(SCI). Ionic imbalance in the injured spinal cord microenvironment (increased Ca2+ influx and K+ efflux), exacerbated oxidative stress from various sources (mitochondrial and ER), and excessive CTSB release all lead to augmented ROS production, thereby activating the NLRP3 inflammasome and triggering pyroptotic signaling. (Colored arrows, Indicate promotion of/causality in related processes; Solid black line, Indicates an increase in ROS; Thin blue arrow: Ion influx/efflux across the cell membrane; Black text, Key biological processes triggering pyroptosis after SCI).

Abbreviations: SCI, Spinal Cord Injury; NADPH, Nicotinamide adenine dinucleotide phosphate; ER, Endoplasmic reticulum stress; ROS, Reactive oxygen species; NOX-2, NADPH oxidase-2; PLA2, Phospholipase A2; CTSB, Cathepsin B; ERO1α, Endoplasmic reticulum oxidoreductase 1 alpha; NLRP3, NOD-like receptor thermal protein domain associated protein 3; ASC, Apoptosis-associated speck-like protein containing a CARD; Caspase-1, Cysteine-aspartic acid protease-1; GSDMD-N, Gasdermin-D N-terminal.

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inflammasome assembly and activation, thereby triggering pyroptosis in the injured spinal cord. In particular, ROS and elevated intracellular Ca^{2+} are indispensable for both inflammasome activation and the downstream execution of pyroptotic cell death.

Connections Between Pyroptosis and Other Pathological Processes

Ample evidence indicates that programmed cell death plays a pivotal role in the pathophysiology of SCI. In SCI, programmed cell death primarily manifests as apoptosis, autophagy, and pyroptosis. Under pathological conditions, the initiation of these pathways can eliminate damaged cells and limit secondary injury; however, their excessive or dysregulated activation can exacerbate tissue damage and impede functional recovery.[89] Moreover, these distinct forms of programmed cell death interact through complex crosstalk rather than operating in isolation, collectively influencing SCI outcomes.

Autophagy and pyroptosis engage in a complex, bidirectional relationship in SCI, and the net impact of autophagy remains debated. After SCI, mitochondrial damage leads to excessive ROS accumulation, which activates the NLRP3 inflammasome and thereby caspase-1–dependent pyroptosis. At the same time, activated caspase-1 cleaves the Toll/IL-1 receptor domain–containing adaptor protein (TRIF), inhibiting TRIF/Toll-like receptor 4 (TLR4)-mediated autophagy.[90] Conversely, autophagy selectively removes damaged mitochondria, ROS, and inflammatory mediators, thus restraining NLRP3 inflammasome activation and suppressing pyroptosis.[91] However, lysosomal membrane rupture releases CTSB, which directly triggers NLRP3 inflammasome activation while simultaneously reducing autophagic flux via Unc-51-like kinase 1(ULK1) phosphorylation at Ser638/758. Studies have shown that melatonin can improve motor function in SCI rats and significantly upregulate the expression of autophagy markers B-cell lymphoma-2-interacting protein (Beclin-1), microtubule-associated protein 1 light chain 3 beta (LC3B), as well as proteins associated with the silent Information regulator 2-related enzyme 1 (SIRT1)/ AMP-activated protein kinase (AMPK) signaling pathway. These effects were abolished by the SIRT1 inhibitor selisistat,[92] indicating that melatonin enhances autophagy and improves motor function in rats via the SIRT1/AMPK pathway, although overly robust autophagy may induce neuronal death.[93] Overall, most researchers believe that autophagy activation may have a protective effect against spinal cord injury. Increased autophagic flux may promote mitophagy and limit ROS production, thereby suppressing NLRP3 inflammasome activation and the secretion of IL-1β and IL-18, ultimately inhibiting pyroptosis. However, the precise role of autophagy in post-SCI pyroptotic cell death remains to be fully elucidated. Its effects may vary across different pathological stages and microenvironments following SCI, making it premature to definitively conclude whether autophagy is beneficial in this context.

Apoptosis and pyroptosis are interconnected, primarily via the caspase family.[94] Specifically, caspase-3, a hallmark of apoptosis, can both inactivate GSDMD, thereby inhibiting pyroptosis, and cleave GSDME to promote pyroptosis.[95] Similarly, caspase-8 mediates pyroptosis by processing GSDMD and GSDME, while also initiating apoptotic signaling.[96] Caspase-1, the central effector of canonical pyroptosis, can paradoxically trigger apoptosis in the absence of its gasdermin substrates.[97] Given that inhibiting apoptosis during the secondary injury phase of SCI is critical, simultaneous suppression of both apoptosis and pyroptosis could be essential for reducing tissue damage and enhancing functional recovery. However, the precise regulatory crosstalk between these two forms of programmed cell death in SCI remains poorly understood. Therefore, elucidating the mechanisms linking apoptosis and pyroptosis represents a vital avenue for future SCI research.

Although pyroptosis plays a critical role in secondary spinal cord injury, its detailed molecular mechanisms and precise relationship to SCI pathology remain unclear. Consequently, therapeutic strategies targeting post-SCI pyroptosis are limited and have largely focused on inhibiting NLRP3 inflammasome activation, blocking inflammasome assembly, and modulating related pathways such as autophagy and apoptosis. To advance pharmacological intervention, future studies must prioritize elucidating how candidate compounds influence these key processes. However, progress has been slow: only a handful of investigations into exosomes and tissue-engineered materials have demonstrated efficacy in regulating pyroptosis. In contrast, bioactive natural products, renowned for their therapeutic potential across diverse diseases, offer a promising avenue for developing novel treatments that inhibit pyroptosis and promote recovery following SCI.

Natural Bioactive Compounds That Modulate Pyroptosis in Spinal Cord Injury

Current pharmacological research in SCI primarily focuses on modulating inflammation and apoptosis, enhancing tissue repair, and promoting neuronal and axonal regeneration. However, pyroptosis remains an underexplored target, particularly with respect to natural bioactive compounds. We searched the CNKI, PubMed, Science Direct, Elsevier, Google Scholar, Wiley, Springer, Scopus and other databases using keywords such as "ethnic medicine," "natural medicine," "traditional Chinese medicine," "herbal medicine," "active ingredient," "spinal cord injury," and "pyroptosis". All pharmacological studies published up to August 2025 were retrieved from the database. After careful screening, studies unrelated to the modulation of pyroptotic processes were excluded. Table 1 summarizes the existing studies on such compounds that modulate pyroptotic mechanisms in SCI.

Table 1 Summary of Natural Drug Active Ingredients with Intervention Effects on SCI Pyroptosis

Active Ingredient	Source	Model	Dose	Outcome Indicators	Effect	Mechanism
Kaempferol[98]	Tea, broccoli, grapefruit, kale, etc.	SD male mice (C5 impaction); BV-2 cells + LPS	In vivo: 25, 50, 100 mg/kg by gavage, once a day (10 d); In vitro: 25, 50, 100 μM (1 h)	Motor function: FLS scores, cylinder test; Histopathology: HE; Inflammation: IL-18, IL-1β, etc; Pyroptosis: NLRP3, caspase-1 p10, GSDMD, etc; Oxidative stress: ROS, MDA, etc.; Pathway: MAPK/NF-κB	Improve spinal cord pathology and restore motor function; Inhibit microglia- and inflammation-associated oxidative stress, and suppress pyroptosis	Inhibit the MAPK/NF-κB and pyroptosis pathways
Piperine[99]	Black pepper	C57BL6J male rat (T8 squeeze)	10, 15 mg/kg intraperitoneal injection, once a day (28 d)	Motor function: BMS, inclined plane test scores; Histopathology: HE, Masson; Inflammation: TNF-α, etc; Autophagy: Beclin-1, p62, etc; Pyroptosis: caspase-1, NLRP3, GSDMD, IL-1β, etc; Oxidative stress: SOD, MDA, etc	Improve spinal cord pathology and restore motor function; Promote autophagy and inhibit oxidative stress, pyroptosis, and inflammation	Activate autophagy in spinal cord tissue cells
Betulinic acid[100]	Birch bark, ziziphus spinosa seeds, platanus species, jujube fruits, etc.	C57BL6J female mouse (T11-T12 impact)	20 mg/kg intraperitoneal injection, once a day (3 d)	Motor function: Footprint analysis, BMS scores; Histopathology: HE, Masson; Autophagy: LC3B, Beclin1, p62, etc; Pyroptosis: caspase-1, NLRP3, GSDMD, etc; Glial scar: SYN/NeuN, MAP2, etc; Oxidative stress: SOD, MDA, etc; Pathway: AMPK/mTOR/TFEB	Improve spinal cord pathology and restore motor function; Inhibit glial scar formation, pyroptosis, inflammation, and oxidative stress; Promote mitophagy	Regulates the AMPK/mTOR/TFEB pathway

(*Continued*)

Table 1 (Continued).

Active Ingredient	Source	Model	Dose	Outcome Indicators	Effect	Mechanism
Hesperetin[101]	Peels of oranges, lemons, grapefruits, etc.	SD male mice (C5 impaction); BV-2 cells (siNrf2 pretreatment) + LPS + ATP	In vivo: 25, 50, 100 mg/kg by gavage, once a day (28 d); In vitro: 10, 20, 50 μM (1 h)	Motor function: rearing test, FLS scores; Pyroptosis: ASC, caspase-1 p10, GSDMD, IL-1β, etc; Oxidative stress: ROS, SOD, etc; Pathway: Nrf2, NLRP3; inflammasome	Inhibit microglial activation; Suppress inflammation, oxidative stress, and pyroptosis, reduce ROS; Protect and promote recovery of injured spinal cord function	Activate Nrf2 and inhibit NLRP3 inflammasome activation
Paeonol[102]	Peony rhizome	SD female mice (T9 compression); BV-2 cells + LPS + ATP	In vivo: 100 mg/kg by gavage, once a day (21 d); In vitro: 4 μM (24 h)	Microglia: CD86, CD206, etc; Histopathology: HE; Inflammation: IL-1β, IL-10, etc; Pyroptosis: ASC, caspase-1, GSDMD, etc; Pathway: NLRP3 inflammasome, RhoA/MAPK, TLR4/MyD88/NF-κB	Improve spinal cord pathology; Suppress inflammation, oxidative stress, pyroptosis, and apoptosis; Promote M2 polarization of microglia	Inhibit the NLRP3 inflammasome, RhoA/MAPK and TLR4/MyD88/NF-κB pathway
Taxifolin[103]	Citrus fruits, grapes, onions, olives, etc.	SD female mice (T9 compression) BV-2 cells, PC12 cells + LPS + ATP	In vivo: 20 mg/kg by gavage, once a day (7 d); In vitro: 25, 50, 75 μmol/L (2 h)	Motor function: BBB scores; Histopathology: HE, LFB; Pyroptosis: NLRP3, GSDMD, caspase-1, IL-1β, etc; Microglia: IBA-1; Oxidative stress: SOD, MDA, etc; Axonal regeneration: NF-200, MAP-2, GAP43, etc; Pathway: PI3K/AKT	Inhibit microglia-associated oxidative stress, pyroptosis, and inflammation; Promote axonal regeneration	Inhibit the PI3K/AKT pathway

Abbreviations: C, Cervical spine; T, Thoracic; LPS, Lipopolysaccharide; BV-2, Mouse microglial cells; PC-12, Adrenal pheochromocytoma cells; SD, Sprague Dawley; ROS, Reactive oxygen species; MAPK, Mitogen-activated protein kinase; NF-κB, Nuclear factor kappa-B; AMPK, Adenosine 5'-monophosphate-activated protein kinase; mTOR, mammalian target of rapamycin; TFEB, T-cell transcription factor EB; Nrf2, nuclear factor erythroid-derived 2-like 2; NLRP3, NOD-like receptor protein 3; TLR4, Toll-like receptor 4; MyD88, Myeloid differentiation primary response gene 88; PI3K, Phosphatidylinositide 3-kinases; AKT, Protein kinase B; FLS, Fibroblast-like synovial cells; SOD, Superoxide dismutase; MDA, Malondialdehyde; NF200, Neurofilament; MAP2, Microtubule-associated protein 2; GAP43, Growth Associated Protein 43; HE, Hematoxylin-eosin; TNF-α, Tumor necrosis factor α; IL, Interleukin; GSDMD, Gasdermin D; BMS, Basso mouse scale; Beclin-1, BCL2-interacting protein; p62, Sequestosome-1; SYN, Synchronize sequence numbers; NeuN, Neuronal nuclei; ASC, Apoptosis-associated speck-like protein containing a CARD; Caspase-1 p10, Caspase-1 isoform alpha p10 subunit; RhoA, Ras homolog gene family member A; IBA-1, Ionized calcium-binding adapter molecule 1.

Kaempferol

Kaempferol is a natural flavonoid abundantly found in fruits and vegetables such as tea, broccoli, grapefruit, and kale, with well-documented anti-inflammatory, antioxidant, antimicrobial, and neuroprotective properties. In neuronal models, kaempferol reduces ROS production by improving mitochondrial function[104] and ameliorates cognitive deficits via modulation of the cholinergic system.[105] Liu et al[98] reported that continuous gavage of kaempferol (25–100 mg/kg) for 10 days reduced the expression of NLRP3, caspase-1 isoform alpha p10 subunit (caspase-1 p10), and GSDMD-N in the brains of 5th cervical vertebra (C5)-injured Sprague–Dawley (SD) rats, thereby inhibiting pyroptotic signaling and decreasing the levels of inflammatory cytokines such as IL-1β and IL-18. In vitro studies using BV2 cells further demonstrated that kaempferol suppressed activation of the mitogen-activated protein kinase (MAPK)–NF-κB pathway, downregulated expression of calcium-binding adaptor molecule 1 (IBA-1), NOX-2, and NOX4, inhibited microglial activation and oxidative stress, and ultimately improved fibroblast-like synovial cells (FLS) scores, ameliorated pathological changes, enhanced performance in the cylinder test, and partially restored motor function in rats. Beyond its anti-pyroptotic effects, kaempferol enhances autophagy, Han et al[106] demonstrated that it mitigates NLRP3-mediated

neuroinflammation in microglia by promoting autophagic flux. Furthermore, studies have shown that kaempferol markedly suppresses the expression of apoptosis-regulating factors, including B-cell lymphoma-2-associated X protein (Bax) and caspase-3, in the hippocampus of rats subjected to the chronic unpredictable mild stress (CUMS) depression model. This inhibition of apoptotic activity may, in turn, attenuate pyroptotic responses.[107]

Kaempferol is widely available, pharmacologically stable, exhibits low adverse effects, and can readily cross the blood–brain barrier, making it a focal point in neuropharmacological research.[108] Studies have shown that orally administered kaempferol is rapidly absorbed in vivo while maintaining its pharmacological activity.[109] In a study by Cao,[110] histological examination of liver and kidney tissues from rats treated with high-dose kaempferol revealed no significant hepatocyte necrosis, inflammatory cell infiltration, disorganization of hepatic lobules, or vascular injury in the liver, and no obvious glomerular or renal vascular lesions in the kidney.

Piperine

Piperine is an alkaloid extracted from pepper. Pharmacological studies have demonstrated that piperine possesses a wide range of activities including anti-inflammatory, antioxidant, antitumor, antiangiogenic, hypoglycemic, hypolipidemic, immunomodulatory, and neuroprotective effects. In the study by H. Zhang et al,[99] they established a 8th thoracic vertebra (T8) spinal cord injury model in male C57BL/6J mice and administered 10–15 mg/kg piperine via intraperitoneal injection for 28 days, they found that piperine improved Basso mouse scale (BMS) and inclined-plane test scores, reduced spinal cord caspase-1, neuron-specific nuclear protein (NeuN), NLRP3, and GSDMD expression, decreased neuronal pyroptosis, increased superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) levels, and lowered malondialdehyde (MDA), IL-1β, IL-18, IL-6, and TNF-α, thereby inhibiting oxidative stress and inflammatory exacerbation. Notably, the study also found that piperine upregulated Beclin-1 and autophagy-related 5 (ATG5) expression, downregulated p62, and enhanced autophagic flux in neurons. Increasing evidence indicates that secondary injury in SCI is closely associated with mitochondrial damage and excessive ROS production. Damaged mitochondria lead to excessive ROS accumulation, thereby inducing NLRP3 inflammasome activation and subsequent caspase-1–dependent pyroptosis.[111] Mitophagy, the selective degradation of damaged mitochondria,[112] can reduce ROS accumulation[113] and may play a pivotal role in inhibiting post-SCI pyroptosis. In terms of ion homeostasis, Pan et al[114] found that in an rat basophil leukemia cells (RBL-2H3) model of deoxyribonucleoprotein (DNP)- immunoglobulin E (IgE)–induced degranulation and DNP- human serum albumin (HAS) stimulation, piperine binds to the cell membrane, attenuates Ca^{2+} influx, and prevents degranulation. Furthermore, whole-cell patch-clamp analysis revealed that piperine exerts anticonvulsant effects by inhibiting Na^{+} channels and blocking Na^{+} influx. Another study showed that piperine treatment in a rat model of myocardial injury significantly downregulated BCL-2-associated X protein/B-cell lymphoma-2(Bax/Bcl-2), caspase-3, and caspase-9 expression, suggesting anti-apoptotic and cytoprotective effects.[115] These findings indirectly suggest that piperine may modulate ion imbalance and apoptosis. However, further SCI-specific studies are required to validate and explore these mechanisms.

Piperine is the first bioavailability enhancer with verified efficacy,[116] and its microemulsification properties can increase blood–brain barrier permeability and systemic bioavailability.[117] However, studies have shown that a dose of 20 mg/kg piperine impairs learning and long-term synaptic plasticity in Alzheimer's disease model rats,[118] while long-term administration at a reduced dose (10 mg/kg for 60 days) disrupts spermatogenesis in male albino rats,[119] suggesting potential neuro- and reproductive toxicity. Additionally, Wu[120] investigated the dose–effect relationship of piperine in SCI and found that an extremely high dose (150 mg/kg) exhibited neurotoxic effects in SCI rats. These findings indicate that future studies should further explore the pharmacological effects of piperine in SCI within a safe and rational dosage range.

Betulinic Acid

Betulinic acid (BA), a lupane-type pentacyclic triterpenoid predominantly isolated from birch bark (Betula spp.) and also present in Ziziphus spinosa seeds, Platanus species, and jujube fruits, possesses broad bioactivities, anti-inflammatory, antimicrobial, antiviral, and anti-aging. It further contributes to antioxidant defense and immune modulation. By targeting T-type (Cav3) and N-type (CaV2.2) calcium channels in dorsal root ganglion neurons, BA inhibits depolarization-

induced Ca^{2+} influx, a key upstream event in both pyroptosis and apoptosis.[121] C. Wu et al[100] confirmed the pyroptosis-inhibitory effects of BA. Studies have found that intraperitoneal injection of 20 mg/kg betulinic acid for 3 days after spinal cord injury (T11-12) in mice reduced the expression of ASC, GSDMD, caspase-1, and NLRP3, thereby inhibiting NLRP3 inflammasome assembly and pyroptosis initiation. This treatment also decreased the expression and secretion of IL-1β, IL-18, and ROS, attenuating local inflammation and oxidative stress, and reducing spinal cord scar formation. Footprint analysis revealed that functional recovery at 28 days post-injury in the BA group was superior to that in the model group, and BMS scores at all post-injury time points were consistently higher in the betulinic acid-treated mice. Interestingly, a study on Alzheimer's disease using APP/PS1 transgenic mice found that betulinic acid can increase the expression of LC3B and Beclin-1 in mouse brain tissue, decrease the expression of sequestosome-1 (p62), and upregulate the expression of BCL2 interacting protein 3 (BNIP3), NIP3-like protein X (NIX), and Parkin, thereby promoting mitophagy, which may help improve symptoms related to spinal cord injury. These effects may be achieved by regulating the AMPK/mammalian target of rapamycin (mTOR)/transcription factor EB (TFEB) signaling pathway. The report also found that AMPK/mTOR activation can promote Ca^{2+} release from neurons in amyloid precursor protein (APP)/presenilin-1 (PS1) transgenic mice through the transient receptor potential mucolipin 1 (TRPML1) channel,[122] which may also be involved in pyroptosis inhibition. Moreover, bioinformatic analyses suggest that betulinic acid may inhibit apoptosis and prevent inflammation spread by suppressing the activity of the proinflammatory target PLA_2,[123] This action could potentially contribute to pyroptosis inhibition, although it has not yet been experimentally validated in the context of SCI.

The poor water solubility of betulinic acid partially limits its bioavailability; however, the development of hydrophilic derivatives such as Boc-lysine–betulinic acid[124] and polymer-encapsulated formulations[125] has effectively enhanced its bioavailability. Betulinic acid is considered a safe and low-toxicity compound, exhibiting minimal cytotoxicity toward normal cells, with very low toxicity to dermal fibroblasts and peripheral blood lymphocytes. Toxicity assessments have shown that intraperitoneal administration of 400 mg/kg in rats produces no significant adverse effects.[126] Furthermore, some studies have reported that betulinic acid may mitigate renal toxicity, potentially through activation of nuclear factor erythroid-derived 2-like 2 (NRF2) and inhibition of the NF-κB pathway.[127]

Hesperetin

Hesperetin (HSP), a flavanone richly found in citrus fruits such as oranges, lemons, and grapefruits, displays potent antioxidant, anti-inflammatory, nitric-oxide–enhancing, antihypertensive, and apoptosis-modulating activities. Beyond its established benefits in insulin resistance, cardiovascular disease, nonalcoholic fatty liver disease, and metabolic syndrome,[128] recent studies have explored its neuroprotective potential in SCI. Zhang et al[129] used a T9-damaged SCI rat model to demonstrate that HSP can reduce free radicals, enhance the activity of endogenous antioxidant enzymes, and inhibit caspase-3-mediated apoptosis, suggesting that its antioxidant effect may also alleviate pyroptosis. More directly, Liu et al[101] showed that oral administration of 25–100 mg/kg HSP for 28 days can improve vertical ability and forelimb motor scores in C5-injured rats by activating the NRF2 pathway: it upregulates HO-1 and NAD(P)H oxidoreductase 1 (NQO1), restores blood–spinal cord barrier integrity, and fosters tissue repair. Concurrently, HSP downregulates spinal IL-1β, IL-18, ASC, caspase-1 p10, and GSDMD-N, thereby inhibiting neuronal pyroptosis, and attenuates microglial activation and oxidative damage. These findings identify NRF2-driven redox modulation as the mechanistic bridge between HSP's antioxidant and anti-pyroptotic actions, underscoring its promise as a multifaceted therapeutic for SCI.

In other contexts, studies have suggested that HSP may exert protective effects by inhibiting neuronal autophagy in Alzheimer's disease model rats.[130] Xu et al[131] reported in an osteoarthritis study that HSP suppresses the threonine protein kinase/protein Kinase B (MST4/AKT) pathway, which may reduce autophagy in mice and thereby improve neural function. Additionally, HSP has been shown to inhibit apoptosis and alleviate inflammation in SCI rats, potentially contributing to its neuroprotective effects.[129]

Hesperetin exhibits very poor water solubility, which limits its bioavailability. Recent studies have shown that the bioavailability and plasma concentration of hesperetin in cocrystal form are significantly higher than those of the raw drug,[132] likely due to improved solubility facilitating absorption. No adverse effects of hesperetin have been reported to date, and it appears to exert hepatoprotective and renoprotective effects through anti-inflammatory and antioxidant

mechanisms, potentially via activation of the NRF2 pathway. However, an SCI-related study using BV2 or primary microglial cells found that hesperetin concentrations below 40 μmol/L showed no significant cytotoxicity, whereas concentrations above 40 μmol/L markedly reduced cell viability.[133]

Paeonol

Paeonol (PAE), a bioactive compound extracted from the traditional Chinese medicinal herbs Cortex Moutan and Paeoniae Radix, exerts multiple pharmacological effects, including antioxidant, anti-inflammatory, antiplatelet, anti-atherosclerotic, and anti-apoptotic activities.[102] Although most research has focused on its cardiovascular benefits, recent studies suggest that PAE may also attenuate secondary damage following SCI. For example, X. Li et al[102] demonstrated in a T9 contusion model of SD rats that continuous gavage administration of 100 mg/kg PAE for 21 days significantly reduced pyroptosis and markers of inflammation, such as ASC, caspase-1, GSDMD-N, IL-1β, IL-18, and TNF-α, in spinal cord tissue. These effects were accompanied by decreased MDA levels, increased glutathione content, and suppression of NLRP3 inflammasome assembly and TLR4/ myeloid differentiation primary response gene 88 (MyD88)/NF-κB pathway activation. Complementary in vitro experiments using BV2 microglia showed that PAE shifts microglial polarization toward an anti-inflammatory M2 phenotype: it downregulates pro-inflammatory mediators (iNOS, TNF-α) while upregulating anti-inflammatory markers (arginase 1(ARG1), IL-10). This dual action, simultaneously inhibiting pyroptosis and promoting M2 polarization, suggests that PAE may facilitate tissue repair and functional recovery after SCI. However, this study did not investigate whether HSP improves motor function in SCI rats.

PAE has been shown to induce arterial vasodilation by inhibiting the activity of voltage-dependent calcium channels.[134] Studies have shown that PAE can reduce Ca^{2+} channel activity in rat ventricular myocytes, thereby lowering heart rate,[135] and can also inhibit voltage-dependent calcium channels in the arterial smooth muscle of hyperlipidemic rats, reducing Ca^{2+} influx.[136] These findings suggest that PAE may have the potential to ameliorate ionic imbalance in SCI and thereby inhibit pyroptotic cell death. Regarding CTSB-related effects, an in vitro enzyme inhibition study in an anti-snake venom context demonstrated that PAE effectively suppresses the PLA_2 activity of *Agkistrodon halys* venom.[137] Since PLA_2 may trigger NLRP3 inflammasome activation by disrupting lysosomal homeostasis during the secondary injury phase of SCI, PAE may also inhibit neuronal pyroptosis in SCI through this pathway.

PAE is also considered a potential therapeutic compound for SCI, exhibiting relatively few adverse effects, with only occasional reports of mild skin reactions. Studies have shown that paeonol exerts effects comparable to cisplatin but with lower cytotoxicity.[138] However, its clinical application is limited by poor water solubility, rapid metabolism, and low bioavailability.[139] The absolute oral bioavailability of paeonol is approximately 20%.[140] Recent strategies, including nanodelivery systems,[141] cyclodextrin derivatives,[142] and microspheres,[143] have improved its bioavailability, but these formulations still suffer from insufficient stability.[144] These findings highlight the need for future development of more stable and effective paeonol formulations.

Taxifolin

Taxifolin is a natural polyphenolic compound extracted from citrus fruits, grapes, onions, and olives, exhibiting uric acid–lowering, hypoglycemic, nephroprotective, hepatoprotective, and cardioprotective effects.[145] Z. Hu et al[103] reported that oral administration of 20 mg/kg of taxifolin for 7 days improved the Basso Beattie Bresnahan (BBB) score of T9 squeezed SD rats, alleviated spinal cord tissue damage, and inhibited activation of the phosphatidylinositide 3-kinases (PI3K)/AKT pathway in the spinal cord. Taxifolin also reduced the expression of NLRP3, GSDMD, ASC, and caspase-1, thereby suppressing pyroptosis, and decreased IBA-1 and MDA levels, mitigating oxidative stress induced by microglial activation. Furthermore, the study found that taxifolin increased the expression of neurofilament 200 (NF-200), microtubule-associated protein 2 (MAP-2), and growth-associated protein 43 (GAP43), while reducing glial fibrillary acidic protein (GFAP) and caspase-3 expression, indicating its potential to promote axonal regeneration, which may be highly beneficial for patients in the chronic phase of SCI. These effects may stem from its blockade of PI3K/AKT-driven mTOR activation and ULK1 Ser333/Ser555 phosphorylation,[103] thereby initiating neuronal autophagy and further inhibiting pyroptosis. Additionally, taxifolin was shown to inhibit phosphorylation of PI3K/AKT pathway proteins in BV2 cells co-stimulated with LPS and ATP.[103]

Taxifolin exhibits very low oral bioavailability (0.17%), but emulsification can effectively increase its bioavailability to over 36%.[146] Although still below the ideal level, it demonstrates potential for therapeutic application. Studies have shown that taxifolin is neither cytotoxic nor proinflammatory, while exhibiting strong anti-glycation activity,[147] to date, no clear reports of adverse effects have been documented, highlighting the need for further investigation into its dose–toxicity relationship.

Current Limitations and Future Directions

Current Limitations

These natural bioactive compounds have demonstrated the ability to repress key pyroptotic signaling molecules, inhibit NLRP3 inflammasome assembly, attenuate oxidative stress by lowering ROS levels, correct ionic imbalances, preserve lysosomal integrity through reduced CTSB release, and modulate both autophagy and apoptosis. However, comprehensive studies on optimal dosing regimens, safety and toxicity profiles, and routes of administration remain lacking, and most candidates are still distant from clinical application. It is worth noting that among the aforementioned natural drug active ingredients, only kaempferol, piperine, and hesperidin have relatively abundant clinical research in terms of clinical translation, with 40, 70, and 33 clinical studies respectively in the PubMed database. However, none of these studies involve the SCI field, and none have been approved in any country for the treatment of SCI or other neurotrauma-related diseases. Nevertheless, given that these compounds have a foundation in clinical research in other fields, and that their administration methods, dosages, and safety profiles are relatively clear and controllable, future clinical translation in the SCI field could begin with kaempferol, piperine, and hesperidin. Furthermore, many pharmacological investigations have not fully elucidated the triggers of pyroptosis or the precise mechanisms of NLRP3 inflammasome activation in SCI. Equally important is ensuring that the severity of secondary injury in animal models faithfully recapitulates human SCI pathology, as this critically affects the translational relevance of experimental therapies. Continued discovery and characterization of natural compounds that target pyroptosis in SCI promise both significant therapeutic potential and deeper insight into the molecular pathways driving injury progression.

Future Research Directions

A translational gap exists between preclinical findings and clinical trial outcomes in the SCI research field, partly due to the heterogeneity and limited reliability of animal model endpoints. For instance, studies on kaempferol, betulinic acid, and taxifolin primarily relied on BBB/BMS scores and footprint analysis, without incorporating more comprehensive behavioral assessments. Future research could include CatWalk™ automated gait analysis, as well as mechanical and thermal nociception tests, to comprehensively evaluate motor, sensory, and autonomic functional recovery.[148] Standardized quantitative criteria for tissue injury and repair should also be established. This could involve quantifying glial scar areas, enumerating specific neuronal populations at the rostral and caudal edges of the lesion, and using flow cytometry or ELISA to assess specific immune cell populations and cytokines within the injury site to evaluate neuroinflammatory status.[149] Moreover, to enhance research quality, future preclinical studies should optimize experimental design under unified endpoints and injury/repair criteria. This includes determining adequate sample sizes based on preliminary data or literature to avoid false-negative or false-positive conclusions, strictly implementing "double-blind" or "triple-blind" protocols, and adhering to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 guidelines to improve transparency and reproducibility.[150]

A systematic preclinical drug development process is essential. Dose–response studies should be conducted prior to efficacy evaluations to determine the maximum tolerated dose, minimum effective dose, and optimal therapeutic window. For example, piperine has been extensively studied for toxicity in animal models. Currently, most studies only assess cytotoxicity in vitro and lack systematic in vivo toxicity and dose–response investigations.Pharmacokinetic considerations should focus on CNS bioavailability. Formulation modifications or alternative administration routes may enhance compound bioavailability. Novel formulations of betulinic acid, paeonol, and hesperetin have been developed and demonstrated improved bioavailability in animal studies. For SCI-specific research, it is critical to verify whether the

compound can effectively cross the BSCB, achieve therapeutic concentrations at the injury site, and maintain sufficient exposure duration to exert its effects.

Combination strategies hold promise for achieving synergistic effects in functional recovery after SCI. The integration of natural compounds with rehabilitation training or cell therapy warrants further investigation. For instance, curcumin combined with treadmill training has been reported to enhance brain-derived neurotrophic factor (BDNF) expression and synaptic plasticity, thereby promoting functional recovery.[151] However, as noted previously, it is essential to employ endpoints that accurately reflect neural and motor function to properly evaluate the efficacy of such combinatorial strategies.

In current SCI research involving pyroptosis, upstream mechanisms of NLRP3 inflammasome activation remain poorly understood. Future studies should further investigate the roles of K^+ efflux, Ca^{2+} influx, and extracellular ATP in initiating and regulating NLRP3 inflammasome assembly following SCI.[152] For diagnostic purposes, multi-omics approaches could be employed to screen for pyroptosis biomarkers, including GSDMD-N, IL-1β, IL-18, and NLRP3 inflammasome components, in cerebrospinal fluid or serum from preclinical models or SCI patients.

Conclusions

Pyroptosis, as a convergence point of the complex pathophysiological mechanisms underlying SCI, may represent a critical therapeutic target for future interventions. Although research on pharmacological treatments for SCI is ongoing, satisfactory functional recovery has yet to be achieved. As an important adjunctive approach, traditional natural products and their bioactive constituents may effectively modulate pyroptosis, thereby mitigating spinal cord tissue damage, promoting neural repair, and enhancing motor function recovery after SCI. Further exploration of these bioactive compounds could facilitate advancements in the clinical management of SCI. However, effective functional recovery is unlikely to rely on a single therapeutic modality; combination strategies may further expand the prospects for future clinical treatment of SCI.

Abbreviations

SCI, Spinal cord injury; BSCB, Blood–spinal cord barrier; ROS, Reactive oxygen species; CTSB, Cathepsin B; CNS, Central nervous system; NLRP3, NOD-like receptor protein 3; GSDMD, Gasdermin D; GSDMD-N, Gasdermin-D N-terminal; AEs, Adverse events; NOX, NADPH oxidase; RNS, Reactive nitrogen species; NF-κB, Nuclear factor kappa-B; TNF-α, Tumor necrosis factor α; CaSR, Calcium-sensing receptor; DUOX1, Dual oxidase 1; ER, Endoplasmic reticulum; TRIF, Toll/IL-1 receptor domain–containing adaptor protein; ULK1, Unc-51-like kinase 1; GSDME, Gasdermin E; SOD, Superoxide dismutase; ZO-1, Zonula Occludens-1; HO-1, Heme oxygenase-1; MDA, Malondialdehyde; BA, Betulinic acid; HSP, Hesperetin; LPS, Lipopolysaccharide; PAE, Paeonol; NF200, Neurofilament; MAP2, Microtubule-associated protein 2; GAP43, Growth Associated Protein 43; GFAP, Glial fibrillary acidic protein; BMS, Basso mouse scale; BBB, Basso Beattie Bresnahan; MST4/AKT, Threonine protein kinase/Protein Kinase B; mTOR, mammalian target of rapamycin; TFEB, Transcription Factor EB; NO, Nitric oxide; NOS, Nitric oxide synthases; iNOS, inducible NOS; IL, Interleukin; AIM2, Absent in melanoma 2; NLRC4, NOD-like receptor family CARD domain-containing protein 4; ASC, Apoptosis-associated speck-like protein containing a CARD; Caspase, Cysteine-aspartic acid protease; ELISA, Enzyme linked immunosorbent assay; cAMP, Cyclic adenosine monophosphate; PDI, protein disulfide isomerase; ERO1α, ER oxidase 1α; mtROS, mitochondrial ROS; BV2, mouse microglial cell; UA, Uric acid; PLA_2, phospholipase A_2; BCL-2, B-cell lymphoma-2; Beclin-1, BCL2-interacting protein; LC3B, microtubule-associated protein 1 light chain 3 beta; SIRT1, silent Information regulator 2-related enzyme 1, AMPK, AMP-activated protein kinase; SD, Sprague–Dawley; C, Cervical spine; T, Thoracic; MAPK, Mitogen-activated protein kinase; CUMS, Chronic unpredictable mild stress; IBA-1, Calcium-binding adaptor molecule 1; RBL-2H3, Rat basophil leukemia cells; DNP, Deoxyribonucleoprotein; IgE, Immunoglobulin E; HAS, Human serum albumin; Bax, BCL-2-associated X protein; p62, Sequestosome-1; BNIP3, BCL2 interacting protein 3; NIX, NIP3-like protein X; TRPML1, Transient receptor potential mucolipin 1; NRF2, Nuclear factor erythroid-derived 2-like 2; NQO1, NAD(P)H oxidoreductase 1; Caspase-1 p10, caspase-1 isoform alpha p10 subunit; TLR4, Toll-like receptor 4; MyD88, Myeloid differentiation primary response gene 88; ARG1, Arginase 1; PI3K, Phosphatidylinositide 3-kinases; ARRIVE, Animal

Research: Reporting of In Vivo Experiments; BDNF, Brain-derived neurotrophic factor; L-O2, Normal human liver cells; CA-074-Me, CA-074 methyl ester; siRNA, small interfering RNA; FLS, Fibroblast-like synovial cells; APP, amyloid precursor protein; PS1, presenilin-1; ATG5, autophagy-related 5.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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