Review

The NLRP3 inflammasome: a potential therapeutic target for traumatic brain injury

Saifudeen Ismael[1], Heba A. Ahmed[1], Tusita Adris[1], Kehkashan Parveen[2], Parth Thakor[1], Tauheed Ishrat[1, 3, *]

Abstract

Although the precise mechanisms contributing to secondary brain injury following traumatic brain injury are complex and obscure, a number of studies have demonstrated that inflammatory responses are an obvious and early feature in the pathogenesis of traumatic brain injury. Inflammasomes are multiprotein complexes that prompt the stimulation of caspase-1 and subsequently induce the maturation and secretion of proinflammatory cytokines, such as interleukin-1β and interleukin-18. These cytokines play a pivotal role in facilitating innate immune responses and inflammation. Among various inflammasome complexes, the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is the best characterized, a crucial role for NLRP3 has been demonstrated in various brain diseases, including traumatic brain injury. Several recent studies have revealed the contribution of NLRP3 inflammasome in identifying cellular damage and stimulating inflammatory responses to aseptic tissue injury after traumatic brain injury. Even more important, blocking or inhibiting the activation of the NLRP3 inflammasome may have substantial potential to salvage tissue damage during traumatic brain injury. In this review, we summarize recently described mechanisms that are involved in the activation and regulation of the NLRP3 inflammasome. Moreover, we review the recent investigations on the contribution of the NLRP3 inflammasome in the pathophysiology of TBI, and current advances and challenges in potential NLRP3-targeted therapies. A significant contribution of NLRP3 inflammasome activation to traumatic brain injury implies that therapeutic approaches focused on targeting specific inflammasome components could significantly improve the traumatic brain injury outcomes.

Key Words: inflammation; interleukin-1β; NLRP3-inflammasome; caspase-1; MCC950; traumatic brain injury

Introduction

Traumatic brain injury (TBI) is one of the leading causes of mortality and disability across the globe, associated with life-threatening public health problems in people of all ages (Ghajar, 2000; Levin and Diaz-Arrastia, 2015). An estimated 2 million people suffer from TBI each year in the US, and, of those, 50,000 die and 80,000 sustain permanent disabilities (Lin et al., 2017). Additionally, there are over 60,000 children hospitalized because of TBI per year in the USA alone (Moreau et al., 2013). Many of those suffer from mild to moderate neurological deficits, which impair their lives irrespective of age or gender (Finnie, 2013). Based on the severity of injury, TBI can be mild, moderate or severe. The current management of TBI focuses on detection and treatment of associated secondary injury, since the damage due to the primary injury is considered to be irreversible (Seule et al., 2015). Primary injury results from the mechanical damage to neuronal tissue that occurs at the time of TBI, including contusion and hemorrhage as a result of shearing, tearing or stretching. Secondary injury then occurs over minutes to days and even months after the initial insult and is characterized by neurochemical, metabolic and behavioral alterations (Kumar and Loane, 2012). The consequences of brain injury can induce behavioral, emotional and cognitive deficiencies, and thus unfavorably affect the quality of life. Rapid and effective treatment is necessary to prevent undesirable complications and promote better recovery. Standard therapy is supportive in nature and focuses on the prevention of secondary complications. New research is ongoing in an effort to elucidate therapeutic strategies for attenuating the devastating consequences of TBI.

The pathomechanisms of TBI stem from mechanical damage that trigger multifactorial secondary cellular responses including disturbances in ionic homeostasis, glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, lipid degradation, diffused axonal injury, initiation of inflammatory and immune responses, and cellular apoptosis (Nizamutdinov and Shapiro, 2017). The initial insult triggers the invasion and migration of immune cells such as macrophages and neutrophils to the injured area, thereby inducing inflammation and edema. These neurochemical events induce the generation of pro-inflammatory mediators such as cytokines, chemokines and prostaglandins, ultimately resulting in blood-brain barrier (BBB) leakage and the development cerebral edema (Kumar

[1]Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA; [2]Department of Biochemistry, Hamdard University, New Delhi, India; [3]Neuroscience Institute, University of Tennessee Health Science Center, Memphis, TN, USA

*Correspondence to: Tauheed Ishrat, PhD, tishrat@uthsc.edu.

https://orcid.org/0000-0002-1342-9869 (Tauheed Ishrat)

How to cite this article: Ismael S, Ahmed HA, Adris T, Parveen K, Thakor P, Ishrat T (2021) The NLRP3 inflammasome: a potential therapeutic target for traumatic brain injury. Neural Regen Res 16(1):49-57.

and Loane, 2012). More than thirty prospective clinical trials for treating TBI have not shown significant promise, as these trials focused on single mechanisms of secondary injury while the mechanisms involved in TBI are clearly multifactorial in nature (Maas et al., 2010).

Although, the exact mechanisms triggering secondary brain injury remain ambiguous, it is suggested that inflammatory responses contribute to the pathophysiology. Post-traumatic neuroinflammation is a central response to the secondary injury that leads to continuous neurodegeneration and neurological impairment. It is characterized by microglial activation, leukocyte recruitment and secretion of inflammatory mediators (Morganti-Kossmann et al., 2007). Gene expression profile analyses in animal models of TBI demonstrated that genes related to inflammation and transcriptional regulators are strongly elevated in the acute phase of injury (Kobori et al., 2002; Natale et al., 2003). Rapid elevation of pro-inflammatory cytokines such as interleukin (IL)-1β, are observed within hours of TBI in both rodents and humans (Li et al., 2011; Lin et al., 2017). The secreted IL-1β can activate other pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) which will further exacerbate the secondary tissue damage (Lu et al., 2005). IL-1β maturation (cleavage of proform) and its release to the systemic circulation is predominantly regulated by the NLRP3 inflammasome, via a newly described mechanism that governs inflammatory responses during secondary tissue injury (Henao-Mejia et al., 2012; Karmakar et al., 2015). Mainly localized in immune cells, the nucleotide oligomerization-like receptor protein 3 (NLRP3) inflammasome is composed of NLRP3, oligomers apoptosis-associated speck-like (ASC) adapter protein and pro-caspase-1, serving as a platform for cleavage of caspase-1 and subsequent IL-1β maturation. According to recent findings, TBI appears to coincide with NLRP3 and ASC oligomerization, inducing the activation of caspase-1 and subsequent IL-1β and IL-18 maturation (**Figure 1**) (Liu et al., 2013; Fan et al., 2017). This finding may be highly significant since NLRP3 has been suggested as a cerebrospinal fluid (CSF) biomarker in TBI patients with poor prognosis (Wallisch et al., 2017). Despite all this evolving research, little has been evaluated with regards to the potential benefit of NLRP3 inhibitors in TBI. Notably, recent studies from our groups and others indicated that pharmacological inhibition of NLRP3 improves neurological outcomes in animal models of TBI. The present review focuses on the mechanism of the inflammatory responses, mediated by the NLRP3 inflammasome and its possible therapeutic potential in the management of TBI.

Search Strategy and Selection Criteria

The most relevant publications included in this article were identified in the PubMed database: https://www.ncbi.nlm.nih.gov/pubmed/. 1) Keywords used for searching (selection criteria): traumatic brain injury, NLRP3 inflammasome, brain, inhibitors, inflammation; neurodegeneration. 2) Dates of searching: 2000–2019.

The Nucleotide Oligomerization-Like Receptor Protein 3 Inflammasome

The innate immune response to cellular stress, infection and tissue injury occurs via pattern- recognition receptors (PRRs) expressed mainly by inflammatory cells such as monocytes, macrophages, neutrophils, and "inflammatory" dendritic cells. PRRs are components of innate immune system, which recognize pathogen-associated molecular patterns (PAMPs), of pathogens, and damage-associated molecular patterns (DAMPs), components of host cells that are released during cellular damage (Schroder and Tschopp, 2010). Toll-like receptors prime the signaling cascade that leads to cellular activation and production of inflammatory mediators, such as TNF, IL-6, IL-8 and type I interferons (IFNs), in response to extracellular signals. Nod- like receptors (NLRs), a group of PPRs, function in the response to danger signals introduced into the host cell cytoplasm. There are more than 20 NLR genes in humans and more than 30 in mice (Ting et al., 2008). These NLRs include NLRP1, NLRP3, NLRC4, NLRC5, NLRP6, NLRP7, NLRP12, as well as the non-NLR inflammasome receptor known as AIM2. Among these, NLRP3 is the most studied inflammasome.

The NLRP3 inflammasome was first described in the Muckle-Wells autoinflammatory disorder, which is associated with spontaneous release of IL-1β (Agostini et al., 2004). The NLRP3 inflammasome is activated by various stimuli and stimulates the activation of caspase-1 (the effector molecule), which sequentially leads to the maturation and release of IL-1β and IL-18, and ultimately potentiates inflammatory responses (Agostini et al., 2004; Sutterwala et al., 2006). NLRP3 contains an amino-terminal-terminal pyrin-only domain (PYD), a conserved central nucleotide binding and oligomerization domain (nucleotide oligomerization or NACHT) and a carboxy-terminal leucine-rich repeat (LRR) domain (Jo et al., 2016). The LRR domain senses PAMPs and other ligands, maintains the NLRP3 in active state, and facilitates protein-protein interactions. The NACHT domain binds with nucleotides, and is vital for protein oligomerization of NLRP3 inflammasome assembly process (Duncan et al., 2007). The PYD domain supports the binding between NLRP and the bipartite adapter ASC via homophilic interactions resulting in the large speck-like structure (Liepinsh et al., 2003). ASC consists of an N-terminal PYD and a C-terminal caspase activation and recruitment domain (CARD). ASC communicate with pro-casapase-1 through the CARD domain (Srinivasula et al., 2002). Under resting state caspase-1 exists as a catalytically dormant precursor, pro-caspase-1. Induction of pro-caspase-1, contributes to the proteolytic maturation of the proenzyme into its active form (Agostini et al., 2004). The activated caspase-1 further induces the maturation and release of pro-inflammatory cytokines IL-1β and IL-18, which belong to the IL-1 family and mediate succeeding immune responses (Keller et al., 2008).

The role of mature caspase-1 is not restricted to secretion of proinflammatory mediators, in fact activated caspase-1 induces inherent inflammatory cell death, known as pyroptosis, which is characterized by cellular swelling, rapid release of intra cellular contents and DNA fragmentation (Bergsbaken et al., 2009; Liu et al., 2018a). Additionally, rupture of the plasma membrane is followed by excessive secretion of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and CX3C-chemokine ligand 3, which may exacerbate pyroptosis as is observed during the acute phase of TBI (Fink et al., 2008; Liu et al., 2018a). Pyroptosis is also known as caspase-1 dependent cell death, and is morphologically and mechanistically distinct from apoptosis and other forms of cell death (Bergsbaken et al., 2009).

## Regulation of Nucleotide Oligomerization-Like Receptor Protein 3 Inflammasome Activation

The NLRP3 inflammasome plays a crucial role in priming inflammatory responses. NLRP3 is induced by a variety of stimuli including pore-forming toxins, extracellular ATP in the presence of various pathogen-associated molecules, uric acid crystals, virus-associated DNA, RNA, asbestos and ultraviolet-B irradiation (Gurcel et al., 2006; Kanneganti et al., 2006; Mariathasan et al., 2006; Martinon et al., 2006; Koo et al., 2008; Muruve et al., 2008). However, the mechanism by which NLRP3 responds to multiple stimuli is elusive. ATP acts as an extra cellular NLRP3 agonist, inducing activation and oligomerization of the inflammasome by inducing K^+ efflux through a P2X purinergic receptor 7 (P2X7)-dependent pore consisting of a pannexin-1 hemichannel. Additionally, NLRP3

can be induced by changes in cellular ionic homeostasis such as low K^+ concentration, Ca^{2+} flux, redox imbalance or mitochondrial dysfunction (Pétrilli et al., 2007; Zhou et al., 2010, 2011; Lee et al., 2012). Several PAMPs and DAMPs induce the activation of the NLRP3 inflammasome, mediated via the thioredoxin-interacting protein (TXNIP) (van Bruggen et al., 2010). Mitochondrial reactive oxygen radicals are the primary stimuli for activation of TXNIP (Kim et al., 2012), and interactions between TXNIP and NLRP3 stimulate the secretion of inflammatory mediators (Zhang et al., 2015). Cytosolic NADPH oxidase 2 (NOX2) also contributes to the activation of the NLRP3 inflammasome through production of reactive oxygen species (Abais et al., 2015). NLRP3 is negatively regulated by nuclear factor erythroid-2 related factor 2 (Nrf2), a redox sensitive transcription factor that regulates expression of cellular antioxidant system. Activators of Nrf2 stimulates the transcription of cellular redox responses that scavenge oxidative damage and results in reduced priming of NF-κB (Jhang and Yen, 2017; Hou et al., 2018). Reduced Nrf2 activity is associated elevated intracellular reactive oxygen species (ROS) level eventually leads to dissociation of TXNIP from thioredoxin and increases the binding of latter to NLRP3 (Jhang and Yen, 2017). Additionally, lysosome destabilization stimulates the activation of NLRP3 by the release of cathepsin B from its contents (Bruchard et al., 2013). Conversely, autophagy acts as negative controller of NLRP3 inflammasome activation (**Figure 1**) (Nakahira et al., 2011).

The intrinsic activity of NLRP3 is modulated by distinct mechanisms. BRCA1-BRCA2-containing complex-3 (deubiquitinating enzyme), double-stranded RNA-dependent protein kinase, death-associated protein kinase 1 and Bruton's tyrosine kinase function as positive regulators of NLRP3 inflammasome activity (Song et al., 2017). A member of the NIMA-related kinase (NEK) family, NEK7 has been proven to directly interact to the LRR domain of NLRP3 and act downstream of K^+ efflux and ROS generation to stimulate the oligomerization of NLRP3 inflammasome (He et al., 2016). K^+ efflux triggers NEK7-NLRP3 assembly which leads to activation of NLRP3 inflammasome, cleavage of pro-caspase-1, and subsequent neuronal injury. NEK7 knockdown in *in vivo* experiments reduces NEK7-NLRP3 binding and attenuates NLRP3 inflammasome activity, pro-caspase recruitment, and pyroptosis in nerve injuries status- post TBI. Additionally, autophagy, microRNAs, CARD-only proteins, pyrin-only proteins and nitric oxide (NO) act as endogenous negative regulators of NLRP3 (Saitoh et al., 2008; Hernandez-Cuellar et al., 2012; Yang et al., 2015; de Almeida et al., 2015).

Role of the Nucleotide Oligomerization-Like Receptor Protein 3 Inflammasome in Traumatic Brain Injury

The main mechanism of secondary brain injury following TBI is known as perilesional edema and is characterized by increased secretion of pro-inflammatory markers, recruitment of innate immune cells into the brain, and activation of local brain astrocytes and microglia (Yi et al., 2019). Accumulating evidence indicates that activation of the NLRP3 inflammasome occurs during the secondary injury of TBI, this has shed a new light on understanding the pathophysiology and development of new strategies for the management of TBI. A temporal relationship between the expression of the NLRP3 inflammasome components and TBI has been reported (Liu et al., 2013). Injury initiated elevated expression of components of the NLRP3-inflammasome, up-regulation of the ASC and caspase-1, and led to maturation and cleavage of IL-1β and IL-18. At the cellular level, NLRP3-inflammasome was identified in neurons, astrocytes, and microglia in the pericontusional cortex. There was a drastic increase in the IL-1β secretion in the pericontusional area at 6 hours post-injury,
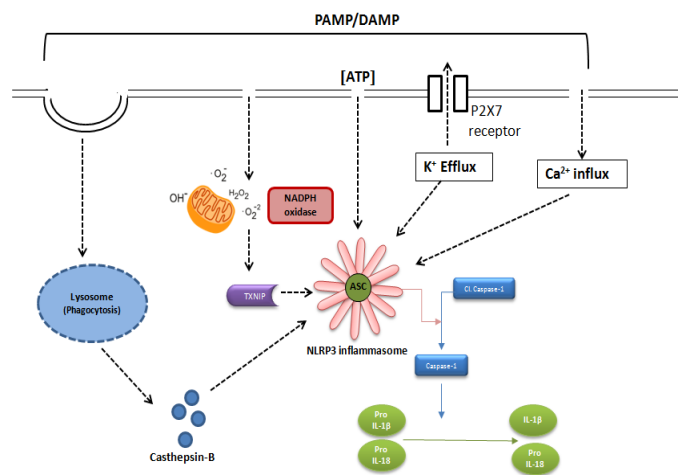
Figure 1 | Factors influencing the assembly and activation of the NLRP3 inflammasome.

NLRP3 is activated by a number of different regulators including various PAMPs and DAMPs. NLRP3 is induced by disturbances in cellular ionic homeostasis such as K^+ efflux and Ca^{2+} influx. Assembly of the NLRP3 inflammasome is mediated by the generation of reactive oxygen free radicals derived from mitochondrial and cytosolic NADPH oxidase. Activated ROS can induce TXNIP, and the interaction between TXNIP and NLRP3 contribute to the activation of NLRP3 inflammasome and the resulting release of inflammatory mediators. Destabilization of phagolysosomes releases lysosomal contents such as cathepsin B, which stimulates the activation of NLRP3. ASC: Apoptosis-associated speck-like protein; DAMP: damage-associated molecular patterns; IL-1β: interleukin-1 beta; IL-18: interleukin-18; NADPH: nicotinamide adenine dinucleotide phosphate; NLRP3: nucleotide oligomerization-like receptor protein 3; PAMP: pathogen-associated molecular patterns; P2RX7: P2X purinoceptor 7; TXNIP: thioredoxin interacting protein.

with a rapid decrease beginning at 24 hours. Conversely, IL-18 protein expression was elevated gradually from 6 hours to 7 days suggesting a role in delayed injury. Supporting data reported a delayed secretion of IL-18 following TBI occurred, which gradually increased over a period of 7 days (Qian et al., 2017). Moreover, protein expression of NLRP3, ASC and caspase-1 continued to increase from 6 hours to 7 days. Acute stimulation of NLRP3 inflammasome components were also observed in a rodent model of penetrating ballistic-like brain injury, which simulates cranial gunshot injury and is associated with progressive tissue loss (Lee et al., 2018). NLRP3 inflammasome activation reached peak at 48 hours after injury followed by the induction of pyroptosis. Initially the inflammasome activation was localized predominantly in neurons and later switched to microglia between 24 and 48 hours after injury. This could be explained by the pyroptotic response of neurons, later stimulate inflammasome activation in neighboring microglia, whereas astrocytes showed delayed inflammasome activation (Lee et al., 2018). At 48 hours after penetrating ballistic-like brain injury, there was a significant rise in the population of primed and ameboid-type microglia expressing inflammasome proteins. These microglia persisted in the injured brain for up to 12 weeks post-injury with continuous neurodegeneration. The possible reason for the discrepancy in the temporal profile of inflammasome expression may be due to variability in extent of brain jury and difference in animal model. The temporal expression pattern of NLRP3 inflammasome components implies that, initial cell death induced by mechanical injury stimulate innate immune response by rapid elevation of NLRP3 and later it is down regulated by activation of protective mechanisms of the body.

One study demonstrated the activation of NLRP3 inflammasome in mediating inflammatory responses in rodent model of blast induced TBI (Ma et al., 2016). They demonstrated that there was increased expression of thioredoxin-interacting protein (TXNIP), a redox sensitive critical regulator of NLRP3 activity, with elevated expression of NLRP3 and activation of caspase-1.

Review

Additionally, there was a higher expression of proinflammatory cytokines including IL-1β and TNF-α in response to blast injury. Administration of propofol (2,6-diisopropyl phenol), a well-known lipid-soluble intravenous anesthetic, was accompanied by attenuation of NLRP3 expression, IL-1β maturation and oxidative stress. Similarly, hyperbaric oxygen treatment attenuated neuroinflammation and brain edema, with accompanied improvements in motor function (Qian et al., 2017). However, the beneficial effect of hyperbaric oxygen treatment on NLRP3 is elusive as it can induce oxidative stress which is known to induce NLRP3 inflammasome (Tschopp and Schroder, 2010). Additionally, the role of NLRP3 inflammasome-associated IL-1β secretion in traumatic cerebral edema has been demonstrated (Erdi et al., 2016). Moreover, treatment with telmisartan, an angiotensin receptor blocker, ameliorated cerebral edema, and subsequently improved neurological outcome which was associated with decreased NLRP3 inflammasome assembly after TBI. The beneficial effect of telmisartan is mediated by inhibition of NLRP3 dependent IL-18 and IL-1β release (Wei et al., 2016). Elevated NLRP3 concentration was found in CSF of children with severe TBI. Elevated expression of NLRP1 was detected in only 11% of children with severe TBI in compared with uninjured controls (Wallisch et al., 2017).

Blast induced TBI is a form of brain trauma due to the use of explosives, for example those associated with war zones and terrorist activities. The resulting secondary damage induces free radical induced TXNIP expression and its downstream NLRP3 inflammasome activation (Fan et al., 2017). Treatment with mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside), a component of traditional Chinese medicine, alleviated brain damage by inhibition of ROS-TXNIP-NLRP3 inflammasome pathway. Supportingly, the contribution of oxidative stress in mediating NLRP3 inflammasome stimulation was recently confirmed. The deletion of NOX-2, a component of NADPH oxidase, a major ROS inducer, attenuated NLRP3 inflammasome activation following TBI and was correlated with reduced interaction between TXNIP and NLRP3. NOX is a family of enzymes particularly devoted to the production ROS. Clinical (autopsy) and experimental studies have confirmed the elevated expression of NOX isoforms following TBI (Zhang et al., 2012; Li et al., 2015). This was localized cortex and hippocampus in animal models (Zhang et al., 2012). Additionally, pharmacological inhibition of NOX is associated with attenuation of apoptosis, inflammation and reduced TXNIP expression (Lucke-Wold et al., 2015). Enhanced oxidative stress, brain water contents (edema) and inflammatory markers that are associated with TBI were attenuated by treatment with resveratrol (Zou et al., 2018). TBI induces down regulation of Nrf2 and subsequent elevation of oxidative and nitrosative stress, which activates neuroinflammation and cellular apoptosis. Deletion Nrf2 function in mice resulted in exacerbated brain damage and inflammatory response in TBI model of fluid percussion injury (Bhowmick et al., 2019). These observations collectively demonstrated that modulation of oxidative stress may attenuate the TXNIP-NLRP3 inflammasome mediated secondary injury after TBI.

Recently, activation of NLRP3 inflammasome was also reported in a rodent model of controlled cortical impact injury (CCI). In this study, treatment with ω–3 fatty acids, alleviated inflammation and behavioral deficits. This response was mediated by the elevated expression of anti-inflammatory β-Arrestin-2 scaffold protein, a downstream molecule of GPR40, which negatively modulates NLRP3 inflammasome activation. The role of NLRP3 has been further confirmed by both genetic and pharmacological approaches. NLRP3 knock out mice demonstrated a preserved cognitive function, less severe damage and less inflammatory response compared to wild-type mice in response to TBI (Irrera et al., 2017).

Additionally, pharmacological inhibition of NLRP3 with BAY 11-7082 revealed more preserved brain structure and reduced edema following TBI. Recently, we have reported that the inhibition of NLRP3 inflammasome components protected mice from TBI induced brain damage in a murine model of CCI (Ismael et al., 2018a). Pharmacological inhibition with highly specific small molecular inhibitor MCC950 protected mice from edema, and resulted in improved neurological function. The study validated both the acute (24 hours) and subacute (72 hours) phases after TBI. Treatment with MCC950 reduced the level of apoptotic markers such as cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP), while causing an upregulation of cell survival molecule protein kinase B. MCC950 also attenuated NLRP3 inflammasome priming by modulation of NFκB p65 expression. However, the study was unable to validate the long term beneficial effect on neurological outcome and structural integrity of the damaged brain. Similar studies were later conducted by another group over an extended period and found elevated NLRP3 inflammasome expression in the peri-contusional cortex and microglia to be the primary source of NLRP3 expression (Xu et al., 2018). Inhibition of NLRP3 inflammasome activation is associated with an improved long term neurological outcome, preserved blood brain barrier integrity, reduced microglial activation, and leukocyte infiltration following TBI. However, MCC950 administration started 12 hours post-TBI did not result in any significant progress. The beneficial effects were evident up to 6 hous post TBI, suggesting that the therapeutic window for MCC950 may be 6 hours. Additionally, it was reported that α2-adrenergic receptor agonist that has been used as sedative hypnotic, analgesic (Fang et al., 2015) has beneficial effects in TBI via NFκB-mediated inhibition of NLRP3 inflammasome, and can attenuate neurological dysfunction (Wang et al., 2018). Dexmedetomidine, a novel α2-adrenergic receptor agonist, has been shown to alleviate post traumatic inflammation through inhibition of the NLRP3 inflammasome.

Dexmedetomidine reduced BBB disruption and cellular apoptosis, and improved neurobehavioral outcomes in the acute phase of TBI (Wang et al., 2018). A recent study evaluated the potential role of receptor-interacting protein 3 (RIP3) in the pathogenesis of TBI (Liu et al., 2018b). RIP3 was found to be upregulated following TBI in parallel with neuroinflammation, impaired neurological and behavioral function. RIP3 ablation improved neurological function and alleviated NLRP3 inflammasome activation, suggesting it may have a regulatory role on the NLRP3 inflammasome. More recently it was demonstrated that NIMA-related kinase 7 (NEK7) mediated NLRP3 inflammasome activation and silencing of NEK7 attenuates NLRP3 inflammasome activation, neurological deficit and pyroptosis following TBI in mouse model of CCI. NEK7 shows a low level of activity under normal circumstances and increased in response to TBI, suggesting its role as the upstream instigator (He et al., 2016). The mechanism of activation of NLRP3 inflammasome in TBI is depicted in **Figure 2**.

TBI is a heterogeneous condition and may result from many different types of insults leading to variable inflammatory responses. There were differences in the temporal profiles of inflammasome activation between the various TBI models, these are likely corresponding to model-specific responses and tissue vulnerability patterns produced by the different models. This makes the comparison more difficult to interpret and limits its translation into clinical trials. Most of the preclinical studies for TBI are well monitored rapid studies, and none of the models simulate the exact clinical condition. Although several pharmacological agents have been validated in experimental studies, none of them have Food and Drug Administration approval for the treatment of TBI. There is an urgent need of specific therapeutic approach targeting multiple mechanism of disease progression in TBI.
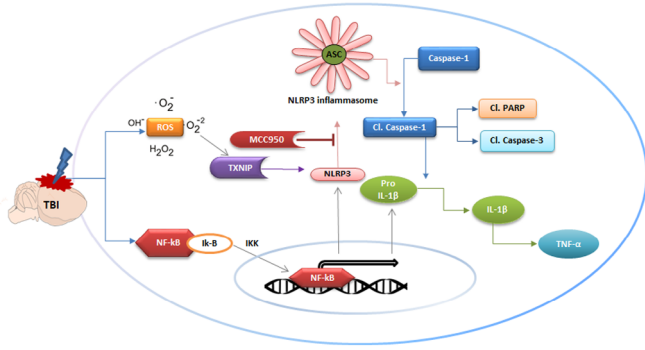
Figure 2 | Mechanism of activation of the NLRP3 inflammasome in TBI, and proposed mode of action of small molecule inhibitor MCC950.

TBI activates the NLRP3 inflammasome by two different mechanisms, first the initial priming signal at the transcriptional level which induces the NF-κB pathway. Secondly, the induction mechanism involving the assembly of the NLRP3 inflammasome complex by direct interaction with TXNIP, a Redox-sensitive regulator. Activated NLRP3 induces the oligomerization of ASC which eventually leads to the assembly of the NLRP3 inflammasome complex. The assembled complex then catalyses the cleavage of Caspase-1 and the subsequent release of IL-1β and inflammatory mediators. Prolonged NLRP3 activation induces proapoptotic molecules such as cleaved PARP and cleaved caspase-3. The specific inhibitor MCC950 blocks NLRP3-induced ASC oligomerization, eventually alleviating the NLRP3-induced neuroinflammation and proapoptotic signaling caused by TBI. ASC: Apoptosis-associated speck-like protein; ATP: adenosine triphosphate; DAMP: damage-associated molecular patterns; IL-1β: interleukin-1 beta; IL-18: interleukin-18; NADPH: nicotinamide adenine dinucleotide phosphate; NLRP3: nucleotide oligomerization-like receptor protein 3; PAMP: pathogen-associated molecular patterns; P2RX7: P2X purinoceptor 7; TXNIP: thioredoxin interacting protein.

Role of Nucleotide Oligomerization-Like Receptor Protein 3 Inflammasome in Other Neurological Diseases and Conditions

NLRP3 mediated inflammasome activation is a pivotal signaling pathway in the innate immune system of the central nervous system (CNS). However aberrant activation of NLRP3 inflammasome signaling has been reported in the pathology of a wide range of neurological diseases. Clinical and *in vivo* studies reported the role of NLRP3 inflammasome in a broad spectrum of microbial infections of the CNS including pneumococcal meningitis, *Staphylococcus aureus* infection (Hanamsagar et al., 2011; Hoegen et al., 2011). Moreover, aberrant expression of NLRP3 inflammosome has been described in viral infections with Japanese Encephalitis virus, influenza virus and human immunodeficiency virus (Hanamsagar et al., 2011; Hoegen et al., 2011; Kaushik et al., 2012; Walsh et al., 2014).

Several studies have validated the pathogenic role of the inflammasome in neurovascular diseases such as Alzheimer's disease (AD), amylotropic lateral sclerosis, multiple sclerosis, ischemic stroke, traumatic injury and microbial infections (Kaushik et al., 2012; Inoue et al., 2012; Geldhoff et al., 2013; Liu et al., 2013; Zhao et al., 2015; Lammerding et al., 2016; Saresella et al., 2016). Inflammatory responses are essential for eliminating invading agents, clearing injured cells and promoting tissue repair. However, prolonged neuroinflammation may lead to further tissue damage and neurovascular dysfunction. The aberrant NLRP3 inflammasome activation is involved in many chronic inflammatory diseases including Alzheimer's disease and aging, which is different from acute neuro inflammation. Chronic inflammation is a low-grade, controlled, chronic systemic inflammatory state and usually asymptomatic, where NLRP3 inflammasome is triggered by metabolic end by products such as urate and cholesterol crystals (Ozaki et al., 2015). In Alzheimer's disease, the increase in Aβ plaques leads to release of DAMPS from neurons. These DAMPs are sensed by NLRP3 inflammasome complex leading to a cascade of events ending in prolonged neuroinflammation (Katsumoto et al., 2018). However, in the case acute brain injury such as TBI and stroke, DAMPs released from injured and necrotic cells and trigger the NF-κB and MAPK signaling pathway through PRRs. These activated pathway contribute to the expression and activation of NLRP3 inflammasome (Alishahi et al., 2019).

Recently we have demonstrated TXNIP mediated enhanced NLRP3 inflammasome activation in cerebral cortex of human AD brain (Li et al., 2019). Increased activation of NLRP3 was reported in intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH), characterized by enhanced production of inflammatory cytokines mediated by reactive oxygen free radicles and the P2X7R pathway (Wang and Doré, 2007; Keep et al., 2012; Feng et al., 2015). Our *in vivo* studies showed that NLRP3 protein expression was found to be increased after experimental ischemic stroke, which was concomitant with an elevated expression of inflammatory mediators IL-1β and IL-18. Pharmacological inhibition of NLRP3 inflammasome improved cerebral ischemia outcomes, as demonstrated by reduced infarction volumes, alleviated neurovascular damage, and the attenuation of inflammatory markers (Ismael et al., 2018b). The significant contribution of NLRP3 in ischemic stroke and spinacord injury were extensively reviewed (Mortezaee et al., 2018; Alishahi et al., 2019).

Nucleotide Oligomerization-Like Receptor Protein 3 Inhibitors in Traumatic Brain Injury

A large number of investigations are warranted for developing a specific NLRP3 inhibitor for the treatment of TBI. Propofol and mangiferin were shown to provide significant inhibition of NLRP3 which is claimed to be responsible for their therapeutic activities in TBI (Fan et al., 2017). Telmisartan, an angiotensin receptor blocker, has also been reported to have NLPR3 antagonistic activity in the state of TBI (Erdi et al., 2016). One recent *in vivo* study demonstrated that BAY11-7082 can attenuate NLRP3 action in the treatment of TBI (Irrera et al., 2017). Treatment with BAY 11-7082 led to a decrease in edema and preserved brain structure in a mouse model of TBI, even though BAY 11-7082 is not a direct inhibitor of NLRP3.

Hyperbaric oxygen treatment inhibited the production of inflammatory markers via inhibition of the NLRP3 inflammasome, but the exact mechanism still needs to be elucidated (Qian et al., 2017). Resveratrol, a nutraceutical drug, also alleviated brain damage following TBI by modifying the expression of NLRP3 inflammasome components, ROS and edema (Zou et al., 2018). The beneficial effect of resveratrol is mediated by NLRP3 attenuation via sirtuin 1 and ROS dependent mechanism. Previously, we have reported that resveratrol inhibits NLRP3 expression through a TXNIP mediated mechanism (Ishrat et al., 2015).

Ketone metabolite β-hydroxybutyrate is known to inhibit inflammatory mediators via NLRP3-specific inhibition, it also inhibits NLRP3-induced ASC oligomerization (Youm et al., 2015). Supplementation with a ketogenic diet specifically inhibits NLRP3 inflammasome activity, without affecting the activation of NLRC4 or AIM2 inflammasomes. β-hydroxybutyrate is bioavailable for vital tissues like the brain and heart under conditions of starvation. Finally, type I IFNs, including IFN-α and IFN-β, are NLRP3-specific inflammasome inhibitors that have been used to inhibit NLRP3 and other inflammasomes in various auto-immune and auto-inflammatory diseases (Shao et al., 2015), however they have not been validated in the setting of TBI.

Artesunate, a derivative of the traditional Chinese phytochemical artemisinin that has been shown to

Review

Table 1 | Summary of studies on the modulation of the NLRP3 inflammasome in TBI

TBI model	NLRP3 modulation	Inference	Reference
Rat model of blast-induced TBI	Propofol, 50 mg/kg, 1 h after explosion	Suppression of enhanced NLRP3 expression with the decreased oxidative stress, cytokine production, and the amelioration of cerebral cortex damage	Ma et al. (2016)
Rat model of blast-induced TBI	Mangiferin 100 mg/kg i.p. 20 min after explosion	Inhibition of TXNIP-NLRP3 inflammasome activation, with accompanied inhibition of oxidative stress and inflammation	Fan et al. (2017)
Mouse model of CCI	NOX2 knockout mice	Attenuation of the expression, assembly, and activity of the NLRP3 inflammasome via modulation of TXNIP NOX2-dependent inflammasome activation contributes to TBI pathology	Ma et al. (2017)
Rat model of CCI	Omega-3 fatty acids (100 mg/kg, twice a week for 6 wk before surgery)	Ameliorated TBI-induced inflammation and behavior deficits by inhibition of NLRP3 inflammasome activation by inducing ARRB2, a negative regulator	Lin et al. (2017)
Mouse model of impact acceleration model of diffused TBI	NLRP3$^{-/-}$ or BAY 11-7082 (20 mg/kg, i.p.), 10 min after injury	Preserved cognitive function, less severe brain damage and attenuation of inflammatory mediators	Irrera et al. (2017)
Mouse model of CCI	MCC950, 50 mg/kg/d	Attenuated inflammasome activation, cerebral edema, and improved neurological function. Modulation of apoptotic signaling and inflammasome priming	Ismael et al. (2018a)
Mouse model of CCI	MCC950 (10 mg/kg body weight, i.p.) daily for the first 3 d and then every other day up to 21 d	Attenuated microglia-derived NLRP3 inflammasome activation and subsequent reduction in brain edema, tissue loss, microglia activation, leukocyte infiltration, BBB disruption, and cell death, and improved long-term neurological function	Xu et al. (2018)
Mouse model of CCI	After injury, animals received 3 d of consecutive dexmedetomidine therapy (25 µg/kg/d)	Inhibited NLRP3 inflammasome pathways by down regulation of NF-κB expression Attenuated neurological dysfunction and brain edema, BBB damage and apoptosis	Wang et al. (2018)
Mouse model of CCI	Hyperbaric oxygen treatment was given for 1 h at 2.0 ATA Once daily for 7 d	Improved motor function and reduced brain edema, with the attenuation of NLRP3 inflammasome activation in microglia	Qian et al. (2017)
Mouse model of CCI	RIP3 knockout	Attenuation of NLRP3 inflammasome activation, RIP3 deficiency alleviated brain edema, lesion volume and neuronal cell death. Improved behavioral performance, and cognitive impairment following TBI	Liu et al. (2018b)
Mouse model of CCI	Artesunate (30 mg/kg) at 1 h after craniotomy	Inhibited NLRP3 inflammasome complex and nuclear factor kappa-B, and modulated the release of neutrophic factors	Gugliandolo et al. (2018)
Rat model of CCI	Dexmedetomidine (20 µg/kg, i.p.) for 4 d	Inhibited NLRP3 inflammasome activation, improved neuronal viability, suppressed microglial activation and improved cognitive function	Zheng et al. (2018)
Rat model of CCI	JC124 (100 mg/kg, i.p.), 4 doses	Inhibited the expression of NLRP3 inflammasome components, decreased cortical lesion volume and improved neuronal survival	Kuwar et al. (2019)
Mouse model of weight drop	Pioglitazone (20 mg/kg, i.p.), daily	Reduced cerebral edema and inflammation by down regulating NLRP3 inflammasome activation. Reduced microglial and astrocyte activation	Yi et al. (2019)
Mouse model of CCI	NIMA-related kinase 7 shRNA, icv following TBI	Down regulation of NLRP3 inflammasome and inflammatory mediators	Chen et al. (2019a)
Rat model of CCI	JC124 (100 mg/kg, i.p.), 4 doses	Inhibited the expression of NLRP3 inflammasome components, decreased cortical lesion volume and improved neuronal survival	Kuwar et al. (2019)

ATA: Atmospheres absolute; BBB: blood-brain barrier; CCI: controlled cortical impact; NIMA related kinase: never in mitosis gene A-related kinase; NLRP3: nucleotide ologomerization-like receptor protein 3; NOX: NADPH oxidase; RIP3: receptor-interacting protein kinase 3; TBI: traumatic brain injury; TXNIP: thioredoxin interacting protein.

have multiple pharmacological actions including anti-neuroinflammatory activity, administration of artesunate decreased the degree of brain injury and lesion volume by inhibiting NLRP3 inflammsome activation and NFκB nuclear translocation in mouse model of CCI (Gugliandolo et al., 2018). Additionally, artesunate protected agaist apoptosis, microglial activation, neuroinflammation and modulated the release of neurotrophic factors. Dexmedetomidine is an alpha 2 adrenergic receptor agonist whose administration in rats subjected to CCI inhibited NLRP3 inflammasome/capasase-1 axis activation, increasing neuronal viability, preventing microglial activation and improving overall cognitive function (Zheng et al., 2018). These responses were comparable to those seen with specific NLRP3 inhibitor BAY-11-7082 in TBI and showed synergetic effect on inhibiting NLRP3 inflammasome activation.

Pioglitazone is a peroxisome proliferator-activated receptor agonist, belongs to the class of thiazolidinediones, a class of drugs used in the management of type 2 diabetes mellitus as it plays a role in increasing insulin sensitivity and promoting adipogenesis and fatty acid uptake. Pioglitazone administration following TBI significantly reduced perilesional cerebral edema through attenuation of NLRP-3 activity and subsequent downstream cytokines and in reducing activation of astrocytes

and microglia (Yi et al., 2019). **Table 1** summarizes the studies on modulation of NLRP3 inflammasome in animal models of TBI.

MCC950, a Novel Selective Nucleotide Oligomerization-Like Receptor Protein 3 Inhibitor

MCC950 is a selective small molecule inhibitor of the NLRP3 inflammasome, and a promising candidate for the treatment of inflammatory diseases (Salla et al., 2016). It is a diarylsulphonylurea-containing compound, specific for NLRP3, but not for AIM2, NLRC4 or NLRP1 inflammasomes, and it blocks NLRP3-induced ASC oligomerization (Coll et al., 2015).

Because of its specificity, MCC950 is less immunosuppressive than other inflammasome inhibitors (Ren et al., 2018) and is orally bioavailable. The beneficial effects of MCC950 have been shown in experimental autoimmune encephalomyelitis and AD, where its ability to cross BBB was verified (Coll et al., 2015; Dempsey et al., 2017). Recently we demonstrated that inhibition of NLRP3 with MCC950 protect mice against TBI. This is the first report validating efficacy of MCC950 in a mouse model of TBI induced by controlled cortical impact (Ismael et al., 2018a). TBI induced activation of

inflammasome, resulted in cerebral edema and impaired neurological function. MCC950 significantly alleviated the expression of inflammasome components such as NLRP3, ASC, cleaved caspase-1 and cleaved IL-1β. It was accompanied with improved neurological function and edema. Inhibition of NLRP3 attenuated the expression of other inflammatory mediator such as NFκB and TNFα. Additionally, MCC950 also provided protection against pyroptotic activation of PARP and caspase-3. Treatment with MCC950 modulated upstream NLRP3 regulator TXNIP. This was the first report addressing the beneficial effects of the selective NLRP3 inhibitor MCC950 on TBI, it was later validated that MCC950 has long term benefit in the mouse model of CCI (Xu et al., 2018). MCC950 attenuated NLRP3 inflammasome activation in the pericontusional area. It reduced brain edema and lesion volume in addition to improved long-term motor and cognitive functions with an activity window of 6 hours. A recent study reported that MCC950 reduces intracerebral hemorrhage via inhibition of the NLRP3 inflammasome (Ren et al., 2018). MCC950 reduced inflammation and attenuated neurological deficits and brain edema in mice after intracerebral hemorrhage was induced by injecting autologous blood or collagenase. Interestingly, treatment with MCC950 also enhanced BBB integrity and attenuated cell death. Recently, Chen et al. (2019b) demonstrated that NLRP3 inhibition along with enhancement of mitophagy with activation of mTOR (mammalian target of rapamycin) with rapamycin improves the neuroprotective effect of NLRP3 inflammasone inhibition following TBI. The proposed mode of action of MCC950 in TBI is depicted in **Figure 2**.

Recently, we have validated the potential benefit of MCC950 in the treatment of secondary brain damage following ischemic/reperfusion injury (Ismael et al., 2018b). Treatment with MCC950 decreased infarct size, edema, hemorrhage, the level of NLRP3 inflammasome components, and alleviated behavioral deficits in a mouse model of middle cerebral artery occlusion. Further long-term studies are required to validate these effects on long term behavioral outcomes. Although pre-clinical studies have validated the therapeutic potential of MCC950 in TBI, there is currently no clinical evidence in this regard. Hence, MCC950 may be a promising pharmaceutical approach for the treatment of TBI in the future.

Glyburide, a sulfonylurea derivative used in management of diabetes mellitus has shown NLRP3 inhibitory action in animal models, however its use is associated with development of hypoglycemia. Randomized clinical trial in patients with moderate and severe TBI shown that oral administration of glyburide is associated with decreased contusion expansion rate and improved neurological outcome (Zafardoost et al., 2016; Khalili et al., 2017). Additionally, intravenous administration of glyburide at low dose in marginally ameliorated lesion volume moderate to severe TBI patients (Eisenberg et al., 2019). Kuwar et al. (2019) has developed a novel small molecule inhibitor JC124 with high specificity for NLRP3 inflammasone. JC124 is structural optimization of glyburide to eliminate hypoglycemic effect. JC124 acts as an NLRP3 inhibitor by blocking ASC aggregation, activation of caspase-1 and release of IL-1β in macrophages and has been shown in rat brain injury models to be neuroprotective in the acute stages following TBI (Kuwar et al., 2019).

Conclusion

The NLRP3 inflammasome, is a multiprotein complex activated in response to brain injury in a time dependent manner. Accumulating evidence demonstrated that the beneficial effects of various non-specific inhibitors in attenuating inflammatory responses is via inhibition of NLRP3 inflammasome in TBI. These beneficial effects are mediated by modulating oxidative stress and the consequent

TXNIP inhibition signify the potential of targeting TXNIP as a possible therapeutic option for the attenuation of NLRP3 inflammasome activation. However, it should be noted that none of the available antioxidants have proven efficacy in clinical trials. Moreover, there is no specific inhibitor for TXNIP. MCC950, specific small molecular NLRP3 inflammasome inhibitor, can cross the BBB and thereby by modulate inflammatory response. Although these studies have clinical implications, limited number of studies validated the beneficial effect of MCC950 in the setting of TBI. Further long-term functional investigation is required to suggest MCC950 as a promising new therapeutic candidate for patients with TBI.

References

Abais JM, Xia M, Zhang Y, Boini KM, Li PL (2015) Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? Antioxid Redox Signal 22:1111-1129.

Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J (2004) NALP3 forms an IL-1beta- processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20:319-325.

Alishahi M, Farzaneh M, Ghaedrahmati F, Nejabatdoust A, Sarkaki A, Khoshnam SE (2019) NLRP3 inflammasome in ischemic stroke: As possible therapeutic target. Int J Stroke 14:574-591.

Bergsbaken T, Fink SL, Cookson BT (2009) Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 7:99-109.

Bhowmick S, D'Mello V, Caruso D, Abdul-Muneer PM (2019) Traumatic brain injury-induced downregulation of Nrf2 activates inflammatory response and apoptotic cell death. J Mol Med Berl Ger 97:1627-1641.

Bruchard M, Mignot G, Derangère V, Chalmin F, Chevriaux A, Végran F, Boireau W, Simon B, Ryffel B, Connat JL, Kanellopoulos J, Martin F, Rébé C, Apetoh L, Ghiringhelli F (2013) Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nat Med 19:57-64.

Chen Y, Meng J, Bi F, Li H, Chang C, Ji C, Liu W (2019a) NEK7 regulates NLRP3 inflammasome activation and neuroinflammation post-traumatic brain injury. Front Mol Neurosci 12:202.

Chen Y, Meng J, Xu Q, Long T, Bi F, Chang C, Liu W (2019b) Rapamycin improves the neuroprotection effect of inhibition of NLRP3 inflammasome activation after TBI. Brain Res 1710:163-172.

Coll RC, Robertson AA, Chae JJ, Higgins SC, Muñoz-Planillo R, Inserra MC, Vetter I, Dungan LS, Monks BG, Stutz A, Croker DE, Butler MS, Haneklaus M, Sutton CE, Núñez G, Latz E, Kastner DL, Mills KH, Masters SL, Schroder K, et al. (2015) A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med 21:248-255.

de Almeida L, Khare S, Misharin AV, Patel R, Ratsimandresy RA, Wallin MC, Perlman H, Greaves DR, Hoffman HM, Dorfleutner A, Stehlik C (2015) The PYRIN domain-only protein POP1 inhibits inflammasome assembly and ameliorates inflammatory disease. Immunity 43:264-276.

Dempsey C, Rubio Araiz A, Bryson KJ, Finucane O, Larkin C, Mills EL, Robertson AAB, Cooper MA, O'Neill LAJ, Lynch MA (2017) Inhibiting the NLRP3 inflammasome with MCC950 promotes non- phlogistic clearance of amyloid-β and cognitive function in APP/PS1 mice. Brain Behav Immun 61:306-316.

Review

Duncan JA, Bergstralh DT, Wang Y, Willingham SB, Ye Z, Zimmermann AG, Ting JP (2007) Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. Proc Natl Acad Sci U S A 104:8041-8046.

Eisenberg HM, Shenton ME, Pasternak O, Simard JM, Okonkwo DO, Aldrich C, He F, Jain S, Hayman EG (2019) Magnetic resonance imaging pilot study of intravenous glyburide in traumatic brain injury. J Neurotrauma 37:185-193.

Erdi F, Keskin F, Esen H, Kaya B, Feyzioglu B, Kilinc I, Karatas Y, Cuce G, Kalkan E (2016) Telmisartan ameliorates oxidative stress and subarachnoid haemorrhage-induced cerebral vasospasm. Neurol Res 38:224-231.

Fan K, Ma J, Xiao W, Chen J, Wu J, Ren J, Hou J, Hu Y, Gu J, Yu B (2017) Mangiferin attenuates blast-induced traumatic brain injury via inhibiting NLRP3 inflammasome. Chem Biol Interact 271:15-23.

Fang B, Li XQ, Bi B, Tan WF, Liu G, Zhang Y, Ma H (2015) Dexmedetomidine attenuates blood-spinal cord barrier disruption induced by spinal cord ischemia reperfusion injury in rats. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol 36:373-383.

Feng L, Chen Y, Ding R, Fu Z, Yang S, Deng X, Zeng J (2015) P2X7R blockade prevents NLRP3 inflammasome activation and brain injury in a rat model of intracerebral hemorrhage: involvement of peroxynitrite. J Neuroinflammation 12:190.

Fink SL, Bergsbaken T, Cookson BT (2008) Anthrax lethal toxin and Salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. Proc Natl Acad Sci U S A 105:4312-4317.

Finnie JW (2013) Neuroinflammation: beneficial and detrimental effects after traumatic brain injury. Inflammopharmacology 21:309-320.

Geldhoff M, Mook-Kanamori BB, Brouwer MC, Troost D, Leemans JC, Flavell RA, Van der Ende A, Van der Poll T, Van de Beek D (2013) Inflammasome activation mediates inflammation and outcome in humans and mice with pneumococcal meningitis. BMC Infect Dis 13:358.

Ghajar J (2000) Traumatic brain injury. Lancet Lond Engl 356:923-929.

Gugliandolo E, D'Amico R, Cordaro M, Fusco R, Siracusa R, Crupi R, Impellizzeri D, Cuzzocrea S, Di Paola R (2018) Neuroprotective effect of artesunate in experimental model of traumatic brain injury. Front Neurol 9:590.

Gurcel L, Abrami L, Girardin S, Tschopp J, van der Goot FG (2006) Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. Cell 126:1135-1145.

Hanamsagar R, Torres V, Kielian T (2011) Inflammasome activation and IL-1β/IL-18 processing are influenced by distinct pathways in microglia. J Neurochem 119:736-748.

He Y, Zeng MY, Yang D, Motro B, Núñez G (2016) NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature 530:354-357.

Henao-Mejia J, Elinav E, Strowig T, Flavell RA (2012) Inflammasomes: far beyond inflammation. Nat Immunol 13:321-324.

Hernandez-Cuellar E, Tsuchiya K, Hara H, Fang R, Sakai S, Kawamura I, Akira S, Mitsuyama M (2012) Cutting edge: nitric oxide inhibits the NLRP3 inflammasome. J Immunol 189:5113-5117.

Hoegen T, Tremel N, Klein M, Angele B, Wagner H, Kirschning C, Pfister HW, Fontana A, Hammerschmidt S, Koedel U (2011) The NLRP3 inflammasome contributes to brain injury in pneumococcal meningitis and is activated through ATP-dependent lysosomal cathepsin B release. J Immunol 187:5440-5451.

Hou Y, Wang Y, He Q, Li L, Xie H, Zhao Y, Zhao J (2018) Nrf2 inhibits NLRP3 inflammasome activation through regulating Trx1/TXNIP complex in cerebral ischemia reperfusion injury. Behav Brain Res 336:32-39.

Inoue M, Williams KL, Gunn MD, Shinohara ML (2012) NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 109:10480-10485.

Irrera N, Pizzino G, Calò M, Pallio G, Mannino F, Famà F, Arcoraci V, Fodale V, David A, Francesca C, Minutoli L, Mazzon E, Bramanti P, Squadrito F, Altavilla D, Bitto A (2017) Lack of the Nlrp3 inflammasome improves mice recovery following traumatic brain injury. Front Pharmacol 8:459.

Ishrat T, Mohamed IN, Pharm B, Pillai B, Soliman S, Fouda AY, Ergul A, El-Remessy AB, Fagan SC (2015) Thioredoxin interacting protein: A novel target for neuroprotection in experimental thromboembolic stroke in mice. Mol Neurobiol 51:766-778.

Ismael S, Nasoohi S, Ishrat T (2018a) MCC950, the selective NLRP3 inflammasome inhibitor protects mice against traumatic brain injury. J Neurotrauma 35:1294-1303.

Ismael S, Zhao L, Nasoohi S, Ishrat T (2018b) Inhibition of the NLRP3-inflammasome as a potential approach for neuroprotection after stroke. Sci Rep 8:5971.

Jhang JJ, Yen GC (2017) The role of Nrf2 in NLRP3 inflammasome activation. Cell Mol Immunol 14:1011-1012.

Jo EK, Kim JK, Shin DM, Sasakawa C (2016) Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 13:148-159.

Kanneganti T-D, Body-Malapel M, Amer A, Park JH, Whitfield J, Franchi L, Taraporewala ZF, Miller D, Patton JT, Inohara N, Núñez G (2006) Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. J Biol Chem 281:36560-36568.

Karmakar M, Katsnelson M, Malak HA, Greene NG, Howell SJ, Hise AG, Camilli A, Kadioglu A, Dubyak GR, Pearlman E (2015) Neutrophil IL-1β processing induced by pneumolysin is mediated by the NLRP3/ASC inflammasome and caspase-1 activation and is dependent on K+ efflux. J Immunol Baltim Md 1950 194:1763-1775.

Katsumoto A, Takeuchi H, Takahashi K, Tanaka F (2018) Microglia in Alzheimer's disease: risk factors and inflammation. Front Neurol 9:978.

Kaushik DK, Gupta M, Kumawat KL, Basu A (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. PLoS One 7:e32270.

Keep RF, Hua Y, Xi G (2012) Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. Lancet Neurol 11:720-731.

Keller M, Rüegg A, Werner S, Beer HD (2008) Active caspase-1 is a regulator of unconventional protein secretion. Cell 132:818-831.

Khalili H, Derakhshan N, Niakan A, Ghaffarpasand F, Salehi M, Eshraghian H, Shakibafard A, Zahabi B (2017) Effects of oral glibenclamide on brain contusion volume and functional outcome of patients with moderate and severe traumatic brain injuries: a randomized double-blind placebo-controlled clinical trial. World Neurosurg 101:130-136.

Kim GS, Jung JE, Narasimhan P, Sakata H, Chan PH (2012) Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. Neurobiol Dis 46:440-449.

Kobori N, Clifton GL, Dash P (2002) Altered expression of novel genes in the cerebral cortex following experimental brain injury. Brain Res Mol Brain Res 104:148-158.

Koo IC, Wang C, Raghavan S, Morisaki JH, Cox JS, Brown EJ (2008) ESX-1-dependent cytolysis in lysosome secretion and inflammasome activation during mycobacterial infection. Cell Microbiol 10:1866-1878.

Kumar A, Loane DJ (2012) Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. Brain Behav Immun 26:1191-1201.

Kuwar R, Rolfe A, Di L, Xu H, He L, Jiang Y, Zhang S, Sun D (2019) A novel small molecular NLRP3 inflammasome inhibitor alleviates neuroinflammatory response following traumatic brain injury. J Neuroinflammation 16:81.

Lammerding L, Slowik A, Johann S, Beyer C, Zendedel A (2016) Poststroke inflammasome expression and regulation in the peri-infarct area by gonadal steroids after transient focal ischemia in the rat brain. Neuroendocrinology 103:460-475.

Lee GS, Subramanian N, Kim AI, Aksentijevich I, Goldbach-Mansky R, Sacks DB, Germain RN, Kastner DL, Chae JJ (2012) The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. Nature 492:123-127.

Lee SW, Gajavelli S, Spurlock MS, Andreoni C, de Rivero Vaccari JP, Bullock MR, Keane RW, Dietrich WD (2018) Microglial inflammasome activation in penetrating ballistic-like brain injury. J Neurotrauma 35:1681-1693.

Levin HS, Diaz-Arrastia RR (2015) Diagnosis, prognosis, and clinical management of mild traumatic brain injury. Lancet Neurol 14:506-517.

Li GZ, Zhang Y, Zhao JB, Wu GJ, Su XF, Hang CH (2011) Expression of myeloid differentiation primary response protein 88 (Myd88) in the cerebral cortex after experimental traumatic brain injury in rats. Brain Res 1396:96-104.

Li L, Ismael S, Nasoohi S, Sakata K, Liao FF, McDonald MP, Ishrat T (2019) Thioredoxin-Interacting Protein (TXNIP) Associated NLRP3 inflammasome activation in human Alzheimer's disease brain. J Alzheimers Dis 68:255-265.

Li Z, Tian F, Shao Z, Shen X, Qi X, Li H, Wang Z, Chen G (2015) Expression and clinical significance of non-phagocytic cell oxidase 2 and 4 after human traumatic brain injury. Neurol Sci 36:61-71.

Liepinsh E, Barbals R, Dahl E, Sharipo A, Staub E, Otting G (2003) The death-domain fold of the ASC PYRIN domain, presenting a basis for PYRIN/PYRIN recognition. J Mol Biol 332:1155-1163.

Lin C, Chao H, Li Z, Xu X, Liu Y, Bao Z, Hou L, Liu Y, Wang X, You Y, Liu N, Ji J (2017) Omega-3 fatty acids regulate NLRP3 inflammasome activation and prevent behavior deficits after traumatic brain injury. Exp Neurol 290:115-122.

Liu HD, Li W, Chen ZR, Hu YC, Zhang DD, Shen W, Zhou ML, Zhu L, Hang CH (2013) Expression of the NLRP3 inflammasome in cerebral cortex after traumatic brain injury in a rat model. Neurochem Res 38:2072-2083.

Liu W, Chen Y, Meng J, Wu M, Bi F, Chang C, Li H, Zhang L (2018a) Ablation of caspase-1 protects against TBI-induced pyroptosis in vitro and in vivo. J Neuroinflammation 15:48.

Liu ZM, Chen QX, Chen ZB, Tian DF, Li MC, Wang JM, Wang L, Liu BH, Zhang SQ, Li F, Ye H, Zhou L (2018b) RIP3 deficiency protects against traumatic brain injury (TBI) through suppressing oxidative stress, inflammation and apoptosis: Dependent on AMPK pathway. Biochem Biophys Res Commun 499:112-119.

Lu KT, Wang YW, Wo YY, Yang YL (2005) Extracellular signal-regulated kinase-mediated IL-1-induced cortical neuron damage during traumatic brain injury. Neurosci Lett 386:40-45.

Lucke-Wold BP, Naser ZJ, Logsdon AF, Turner RC, Smith KE, Robson MJ, Bailes JE, Lee JM, Rosen CL, Huber JD (2015) Amelioration of nicotinamide adenine dinucleotide phosphate-oxidase mediated stress reduces cell death after blast-induced traumatic brain injury. Transl Res 166:509-528.

Ma J, Xiao W, Wang J, Wu J, Ren J, Hou J, Gu J, Fan K, Yu B (2016) Propofol inhibits NLRP3 inflammasome and attenuates blast-induced traumatic brain injury in rats. Inflammation 39:2094-2103.

Ma MW, Wang J, Dhandapani KM, Brann DW (2017) NADPH oxidase 2 regulates NLRP3 inflammasome activation in the brain after traumatic brain injury. Oxid Med Cell Longev 2017:6057609.

Maas AIR, Roozenbeek B, Manley GT (2010) Clinical trials in traumatic brain injury: past experience and current developments. Neurother J Am Soc Exp Neurother 7:115-126.

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440:228-232.

Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440:237-241.

Moreau JF, Fink EL, Hartman ME, Angus DC, Bell MJ, Linde-Zwirble WT, Watson RS (2013) Hospitalizations of children with neurologic disorders in the United States. Pediatr Crit Care Med 14:801-810.

Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossmann T (2007) Modulation of immune response by head injury. Injury 38:1392-1400.

Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A (2018) Inflammasome: Its role in traumatic brain and spinal cord injury. J Cell Physiol 233:5160-5169.

Muruve DA, Pétrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, Parks RJ, Tschopp J (2008) The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature 452:103-107.

Nakahira K, Haspel JA, Rathinam VAK, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, Fitzgerald KA, Ryter SW, Choi AMK (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12:222-230.

Natale JE, Ahmed F, Cernak I, Stoica B, Faden AI (2003) Gene expression profile changes are commonly modulated across models and species after traumatic brain injury. J Neurotrauma 20:907-927.

Nizamutdinov D, Shapiro LA (2017) Overview of traumatic brain injury: an immunological context. Brain Sci doi: 10.3390/brainsci7010011.

Ozaki E, Campbell M, Doyle SL (2015) Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives. J Inflamm Res 8:15-27.

Pétrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J (2007) Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Differ 14:1583-1589.

Qian H, Li Q, Shi W (2017) Hyperbaric oxygen alleviates the activation of NLRP-3-inflammasomes in traumatic brain injury. Mol Med Rep 16:3922-3928.

Ren H, Kong Y, Liu Z, Zang D, Yang X, Wood K, Li M, Liu Q (2018) Selective NLRP3 (pyrin domain-containing protein 3) inflammasome inhibitor reduces brain injury after intracerebral hemorrhage. Stroke 49:184-192.

Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, Tanaka K, Kawai T, Tsujimura T, Takeuchi O, Yoshimori T, Akira S (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 456:264-268.

Salla M, Butler MS, Pelingon R, Kaeslin G, Croker DE, Reid JC, Baek JM, Bernhardt PV, Gillam EMJ, Cooper MA, Robertson AAB (2016) Identification, synthesis, and biological evaluation of the major human metabolite of NLRP3 inflammasome inhibitor MCC950. ACS Med Chem Lett 7:1034-1038.

Saresella M, La Rosa F, Piancone F, Zoppis M, Marventano I, Calabrese E, Rainone V, Nemni R, Mancuso R, Clerici M (2016) The NLRP3 and NLRP1 inflammasomes are activated in Alzheimer's disease. Mol Neurodegener 11:23.

Schroder K, Tschopp J (2010) The inflammasomes. Cell 140:821-832.

Seule M, Brunner T, Mack A, Hildebrandt G, Fournier JY (2015) Neurosurgical and intensive care management of traumatic brain injury. Facial Plast Surg 31:325-331.

Shao BZ, Xu ZQ, Han BZ, Su DF, Liu C (2015) NLRP3 inflammasome and its inhibitors: a review. Front Pharmacol 6:262.

Song L, Pei L, Yao S, Wu Y, Shang Y (2017) NLRP3 inflammasome in neurological diseases, from functions to therapies. Front Cell Neurosci 11:63.

Srinivasula SM, Poyet JL, Razmara M, Datta P, Zhang Z, Alnemri ES (2002) The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. J Biol Chem 277:21119-21122.

Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, Bertin J, Coyle AJ, Galán JE, Askenase PW, Flavell RA (2006) Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity 24:317-327.

Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, Flavell RA, Girardin SE, Godzik A, Harton JA, Hoffman HM, Hugot JP, Inohara N, Mackenzie A, Maltais LJ, Nunez G, Ogura Y, Otten LA, Philpott D, Reed JC, et al. (2008) The NLR gene family: a standard nomenclature. Immunity 28:285-287.

Tschopp J, Schroder K (2010) NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? Nat Rev Immunol 10:210-215.

van Bruggen R, Köker MY, Jansen M, van Houdt M, Roos D, Kuijpers TW, van den Berg TK (2010) Human NLRP3 inflammasome activation is Nox1-4 independent. Blood 115:5398-5400.

Wallisch JS, Simon DW, Bayır H, Bell MJ, Kochanek PM, Clark RSB (2017) Cerebrospinal fluid NLRP3 is increased after severe traumatic brain injury in infants and children. Neurocrit Care 27:44-50.

Walsh JG, Reinke SN, Mamik MK, McKenzie BA, Maingat F, Branton WG, Broadhurst DI, Power C (2014) Rapid inflammasome activation in microglia contributes to brain disease in HIV/AIDS. Retrovirology 11:35.

Wang D, Xu X, Wu YG, Lyu L, Zhou ZW, Zhang JN (2018) Dexmedetomidine attenuates traumatic brain injury: action pathway and mechanisms. Neural Regen Res 13:819-826.

Wang J, Doré S (2007) Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 27:894-908.

Wei X, Hu CC, Zhang YL, Yao SL, Mao WK (2016) Telmisartan reduced cerebral edema by inhibiting NLRP3 inflammasome in mice with cold brain injury. J Huazhong Univ Sci Technolog Med Sci 36:576-583.

Xu X, Yin D, Ren H, Gao W, Li F, Sun D, Wu Y, Zhou S, Lyu L, Yang M, Xiong J, Han L, Jiang R, Zhang J (2018) Selective NLRP3 inflammasome inhibitor reduces neuroinflammation and improves long-term neurological outcomes in a murine model of traumatic brain injury. Neurobiol Dis 117:15-27.

Yang Z, Zhong L, Xian R, Yuan B (2015) MicroRNA-223 regulates inflammation and brain injury via feedback to NLRP3 inflammasome after intracerebral hemorrhage. Mol Immunol 65:267-276.

Yi HJ, Lee JE, Lee DH, Kim YI, Cho CB, Kim IS, Sung JH, Yang SH (2019) The role of NLRP3 in traumatic brain injury and its regulation by pioglitazone. J Neurosurg 27:1-9.

Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, D'Agostino D, Planavsky N, Lupfer C, Kanneganti TD, Kang S, Horvath TL, Fahmy TM, Crawford PA, Biragyn A, Alnemri E, Dixit VD (2015) The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 21:263-269.

Zafardoost P, Ghasemi AA, Salehpour F, Piroti C, Ziaeii E (2016) Evaluation of the effect of glibenclamide in patients with diffuse axonal injury due to moderate to severe head trauma. Trauma Mon 21:e25113.

Zhang QG, Laird MD, Han D, Nguyen K, Scott E, Dong Y, Dhandapani KM, Brann DW (2012) Critical role of NADPH oxidase in neuronal oxidative damage and microglia activation following traumatic brain injury. PLoS One 7:e34504.

Zhang X, Zhang JH, Chen XY, Hu QH, Wang MX, Jin R, Zhang QY, Wang W, Wang R, Kang LL, Li JS, Li M, Pan Y, Huang JJ, Kong LD (2015) Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation. Antioxid Redox Signal 22:848-870.

Zhao W, Beers DR, Bell S, Wang J, Wen S, Baloh RH, Appel SH (2015) TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. Exp Neurol 273:24-35.

Zheng B, Zhang S, Ying Y, Guo X, Li H, Xu L, Ruan X (2018) Administration of Dexmedetomidine inhibited NLRP3 inflammasome and microglial cell activities in hippocampus of traumatic brain injury rats. Biosci Rep doi: 10.1042/BSR20180892.

Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11:136-140.

Zhou R, Yazdi AS, Menu P, Tschopp J (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature 469:221-225.

Zou P, Liu X, Li G, Wang Y (2018) Resveratrol pretreatment attenuates traumatic brain injury in rats by suppressing NLRP3 inflammasome activation via SIRT1. Mol Med Rep 17:3212-3217.

C-Editors: Zhao M, Li JY; T-Editor: Jia Y