Original Article
Inflammasome in drug abuse

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Abstract: Drug abuse disorders refer to a set of related negative health implications associated with compulsive drug seeking and use. Because almost all addictive drugs act on the brain, many of them cause neurological impairments after long-term abuse. Neuropathological studies have revealed a widespread impairment of the cellular elements. As the key components to limit the damage of neural cells, CNS immune system is also found affected by these drugs, directly or indirectly. It has been shown that drugs of abuse alter neuroimmune gene expression and signaling. Growing studies on neuroimmune factors further demonstrate their indispensable role in drugs-induced neurotoxicity. As an important proinflammatory intracellular receptor, inflammasome is activated in many neurodegenerative diseases in response to a broad range of damage-associated molecular patterns (DAMPs) signals. In the cases of drug abuse, especially in those with comorbid of HIV infection and sustained pain, inflammasome activation significantly promotes the neuroinflammation-associated toxicities. To understand inflammasome in drug-associated neurotoxic activity, we reviewed the role played by inflammasome in drug abuse-induced microglial neurotoxicity and evaluated the potential of inflammasone as a therapeutic target for drug abuse disorders based on recent development of various selective small-molecular inflammasome inhibitors.

Keywords: Drug abuse, microglia, neuroinflammation, NLRP3, proinflammatory cytokines

An overview of inflammasome in the central nervous system

In a healthy central nervous system (CNS), due to the physical isolation of blood brain barrier (BBB), the peripheral immune cell infiltration and free passage of blood molecules are highly restricted [1-4]. For this reason, the CNS resident innate immune system is primarily response to invading pathogens and/or tissue damage [5]. Increasing attention has in recent years focused on Nod-like receptors (NLRs), an cytoplasmic pattern recognition receptor (PRRs) that is responsible for processing and release of IL-1β and IL-18 [6]. Up to now, 22 members of NLRs have been found in human and 34 in mice, which can be divided into four subfamilies based on their different N-terminal regions (NLRA, NLRB, NLRC, and NLRP) [7, 8]. As important cytosolic sensors, the NLRs are responsible for the reorganization of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [9]. Many studies have been carried out to determine the roles of NLRs in neuropathogenesis. The subfamily NLRPs (1, 3, 10, 12) have been shown to be associated with the development of neurodegenerative diseases including multiple sclerosis (MS), Alzheimer’s disease (AD) and Parkinson’s disease (PD). Kong et al analyzed the database of an RNA-sequencing transcriptome and splicing database on the expression levels of NLRs in brain neural cells and they found among all cell types in the brain expressing NLRs, the NLRP3 is primarily expressed in the microglia [8]. As drug abuse-induced inflammasome activation is primarily medicated by NLRP3, this review focuses on the role of NLRP3 in microglia.

The NLRP3 inflammasomes are multimeric protein complexes composed of cytosolic sensor NLRP3, bridge protein apoptosis-associated speck-like protein containing a CARD (ASC), and cysteine protease caspase-1 [10]. In response to stimuli, NLRP3 will recruit the ASC protein and serve as caspase-1-activating scaffold. The
inactive pro-caspase-1 will oligomerize and be autoproteolytically cleaved into the active form [10]. Activation of caspase-1 will directly induce the processing and release of IL-1β and IL-18.

In contrast to other NLRs that require direct binding with their activating ligand, the interaction of ligands and NLRP3 is more complex [11]. There is an expanding list of NLRP3 ligands, which are mostly structure and function unrelated [12]. Currently, the exact mechanisms of how the NLRP3 protein interact with such a wide variety of ligands are still largely unknown. However, certain common patterns of activating stimulus have been identified. It is widely accepted that the full activation of NLRP3 requires two independent signals. The first priming signal is stimulated by NF-κB pathway to upregulate the major component (NLRP3) and substrate (pro-IL-1β) of inflammasome [13]. Emerging evidences indicate that the priming process is more complicated, which involves post-translation regulation of NLRP3 and ASC protein [14-18]. As a prototypical first activation signal, LPS was found to prime NLRP3 both in transcriptional and post-translational manners [19]. For this reason, even in researches related to CNS sterile inflammation, LPS is also widely applied as the first priming signal to investigate inflammasome activation [20-22]. After priming, distinct signals are required to active NLRP3 and thus promote the assembly of inflammasome complex. The potential involved pathways have been extensively studied and three activation models were introduced: potassium flux through ion channels, lysosomal membrane destabilization and release of cathepsin, and mitochondrial damage [10].

In the context of traumatic injury, neurodegenerative disease, and long-term drug abuse induced neurotoxicity, the CNS resident immune system primarily deals with self-derived “sterile insults” released from damaged neuron or other glial cells. The result of the immune cells activation can be either beneficial or detrimental. It is important to determine the timing of engagement and specific downstream inflammatory pathways that directly associated with neuropathology. Otherwise, non-specific immune suppressive therapy will also eliminate the inflammatory cascade that supports healing. Thus, our ultimate research goal is to promote the resolution of inflammation by dampening the specific signaling pathways tied to neurotoxicity. Abundant evidence indicate that NLRP3 inflammasome is actively involved in chronic sterile CNS inflammation and lead to detrimental consequences [20, 21, 23-29]. The critic role of NLRP3 inflammasome in promoting neuronal damage was further demonstrated in traumatic-induced injury [30]. In both neurodegenerative disease and acture neuronal injury, the DAMPs will be released and quickly initiate a cascade of danger-associated intracellular signaling, which activates the NLRP3 inflammasome that orchestrate the inflammatory signaling in response to neuronal danger signals. One of the most potent effect of NLRP3 inflammasome activation is through processing and release of IL-1β and IL-18 that are critical amplifiers of the innate immune response to CNS damage. The excessive release of pro-inflammatory cytokine exacerbates excitotoxicity-induced neuronal damage [31-33], and in turn, promote the DAMPs-induced inflammasome activation. This vicious cycle can be further fueled by neuropathogenic factors such as amyloid-β or α-synuclein. In general, NLRP3 inflammasome signaling is a specific inflammatory pathway tightly associated with initiation and maintenance of neurotoxicity. Thus, targeting inhibition on NLRP3 inflammasome activation might be a promising therapeutic strategy.

Recently, novel specific small-molecular inhibitor (MCC950) has been developed and successfully reduced the IL-1β production in vivo [34]. The specific inhibition of NLRP3 inflammasome not only delayed the onset of experimental autoimmune encephalomyelitis (EAE), but also attenuated the severity of disease. Administration of MCC950 to APP/PS1 mice (animal model of Alzheimer’s disease) significantly reduced the amyloid-β accumulation and thus, significantly improved the cognitive function. The enhanced clearance of amyloid-β is achieved by increased phagocytosis of microglia, which dampen the neuroinflammation-induced neurotoxicity [35]. In addition to small-molecular inhibitor, microRNA that negatively regulates NLRP3 expression was also identified [36]. Overexpression of miR-223 reduced the erythrocyte lysis associated microglia activation and neuronal damage, which suggests a protective role of miR-223 in intracerebral hemorrhage.
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Like traumatic injury and neurodegenerative disease, both neuronal autonomous and non-autonomous cell death contribute to the long-term drug abuse-induced neurotoxicity. The microglial NLRP3 is activated in response to DAMPs release, promoting the proinflammatory-induced secondary neuronal damage. By introducing specific NLRP3 inhibition strategy, the auto-amplification loop between neuronal damage and sterile inflammation may be cut off. In next part, we will review the current research evidence that supports the roles of NLRP3 activation in neuronal dysfunction induced by long-term abuse of drugs.

Inflammasome in alcohol abuse

According to the 2015 National Survey on Drug Use and Health (NSDUH), 6.2 percent of people that ages 18 and older suffered from the Alcohol Use Disorder (AUD) in the USA. There was around 88000 people died with alcohol-related reason (Alcohol and Public Health: Alcohol-Related Disease Impact. Centers for Disease Control and Prevention). Because alcohol molecule is small and amphipathic, it can freely cross the BBB. Long-term abuse of alcohol has been reported to be associated with many negative impacts on normal neurological function, including habit formation, decision making, stress, and reward [37]. Animal studies indicated that chronic use of alcohol induced microglia and astrocyte activation accompanied with increased proinflammatory cytokines, which inhibited the neurogenesis and induced long-term behavioral alterations [38-42]. Two important alcohol related neurotoxic mechanisms have been identified including the release of proinflammatory factors and the ROS activation [43]. Overactivated microglia are considered primarily responsible for elevated neuroinflammatory signaling and contribute to development of neurological deficit [44, 45]. In contrast, anti-neuroinflammatory drug minocycline and doxycycline significantly decreased the alcohol consumption [46, 47]. These promising therapeutic effects of anti-neuroinflammatory strategy push researchers to further investigate the molecular mechanisms of alcohol-induced neuroinflammation.

IL-1 related signaling pathway has been well-studied in alcohol-associated neurological impairment. In human postmortem brain tissue, expression of IL-1β and inflammasome proteins (NLRP3 and NLRP1) are significantly upregulated in hippocampal area [48]. The administration of IL-1 receptor antagonist significantly reduced the acute alcohol-induced sedation and promote the recovery from alcohol-induced motor impairment [49]. It is well-accepted that IL-1 signaling is regulated both by TLR4-NF-κB pathway and caspase-1-induced maturation process, which is priming and activation signal of inflammasome [50]. For this reason, the roles of inflammasome in alcohol-induced toxicity were extensively studied. As the important priming signal of inflammasome, TLR4 was found upregulated in microglia and played a pivotal role in alcohol-induced proinflammatory cytokine production and psychological impairments [38, 51-54]. In addition to TLR4, another important inflammasome priming signal, high mobility group box-1 (HMGB1), has also been found released from damaged neuron [55, 56]. Further studies indicate that alcohol induced the acetylation and phosphorylation on HMGB1, which is highly associated with its release [57-59]. Correspondingly, the expressional level of receptors (TLR2, TLR4, TLR9, and RAGE) of HMGB1 were also found elevated in response to alcohol consumption [59]. There is evidence indicating that NLRP3 activation in macrophage/monocyte will, in turn, stimulate the release of HMGB1 to amplify the inflammatory signaling [60]. However, whether such vicious cycle exists in alcohol-induced neuroinflammation has not been investigated. As for the second activation signal of the inflammasome, current studies suggest that alcohol-induced mitochondrial dysfunction and oxidative stress contribute to the inflammasome activation in astrocyte and neuronal progenitor cells [61, 62]. Taken together, both the first priming signal and the second activation signal were proved to be induced by alcohol. To fully characterize the inflammasome activation in alcohol-induced neuroinflammation, a study performed on mice with both pharmacological and genetic manipulation of an essential component of the NLRP3 inflammasome. After administrated with 5% of ethanol for 5 weeks, mice in control group increased in expressional level of NLRP1, NLRP3, ASC, and proinflammatory cytokines, suggesting a functional role of alcohol as the first priming signal. On the other hand, the increased caspase-1 activity and release of IL-1β proved that alcohol
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Gut-liver-brain axis is another promising but still less studied direction in inflammasome research on alcohol abuse. Because alcohol consumption could impair the integrity of BBB, it has been hypothesized that the peripheral endotoxin might cross into the brain [63, 64]. On the other hand, it is well-accepted that alcohol disrupts the intestinal epithelium integrity, which promotes the translocation of intestinal microbiome [65-67]. Thus, it is reasonable to hypothesize that alcohol increases endotoxin levels in the circulation and promotes the entrance of endotoxins into the brain. However, recent evidence suggests that endotoxin level did not change after 5 weeks of alcohol administration, which ruled out this possibility [59]. This might attribute to the fact that, in a healthy individual, the circulated endotoxin would be kept in check by the interaction of multiple organs [68]. However, it is still unknown if translocated endotoxin could escape the surveillance of immune system in a late stage of alcohol abuse, which is featured by a persistent systemic inflammation and liver detoxification impairment. In that case, it is still possible that gut-derived endotoxin will cross into the brain and prime the alcohol-induced inflammasome activation. Another potential connection between gut and brain in the context of alcohol abuse is through the liver damage. It has been found that alcohol-induced translocation of endotoxin will initiate the inflammasome-related proinflammatory response in liver [69]. The activation of inflammasome not only plays a central role in alcoholic liver disease but also promotes the releasing of liver DAMPs into the blood [70, 71]. Because the liver is considered as a major source of inflammatory cytokines that release into the serum [72], liver inflammasome activation induced by gut-derived endotoxin will further amplify the systematic inflammation. Once these circulated proinflammatory cytokines enter the brain tissue and activate the NF-κB pathway, the CNS inflammasome will be primed to be activated [73]. Lastly, whether gut-liver-brain axis was achieved by the liver-potentiated generalized immune response and subsequent neuroinflammation is worth investigating. The inflammasome activation both in liver and brain might serve as a converging signaling that provides a promising therapeutic target to dampen the inflammatory cross-interaction in systemic level.

Inflammasome in psychostimulants abuse

Cocaine and methamphetamine (Meth) are two primary abused psychostimulants in the United States. Cocaine is a psychostimulant drug that binds to dopamine transporter and inhibits its reuptake of synaptic dopamine. Compared to cocaine, methamphetamine not only blocks the reuptake but also promotes the dopamine release. They are both highly addictive and neurotoxic after long-term abuse. Accumulating evidence suggest that neuroinflammation underlies the mechanisms of neurotoxicity and provides a promising target. To better understand the mechanisms of cocaine- and Meth-related neuroinflammation, latest researches on inflammasome activation will be reviewed. Also, whether inflammasome-related signaling serves as a converging pathway that mediates the synergistic neurotoxic effect of HIV-1 infection with stimulant abuse will also be discussed.

In mice administrated with cocaine, NF-κB signaling was found to be activated and associated with structural changes that mediate the
drug reward-based learning [74, 75]. In consistent with this, upregulation of multiple proinflammatory mediators and microglia activation were detected in cocaine abused animal models [76-78]. It has been suggested the proinflammatory signaling activation is due to the dysregulated redox status after cocaine administration [79-81]. Although with the activation of the first signal (NF-κB signaling) and the second signal (oxidative stress), there is little study indicate the inflammasome activation in the brain after exposure to the cocaine. In cultured microglia, cocaine stimulates the proinflammatory release mediated through the ER stress-autophagy and ER stress-TLR2 axes [82, 83]. However, given the fact that ER stress is also a well-accepted activating signal for NLRP3 inflammasome activation [84, 85], there is no further study performed to investigate the role of the inflammasome in cocaine-induced microglial activation.

On the other hand, cocaine downregulates the expression of tight junction while it upregulates the expression of the brain endothelial adhesion molecules and CCL2 [86, 87]. Therefore, cocaine not only impairs the integrity of BBB but also promotes the transmigration of monocyte. In HIV-1 infected patients, cocaine abuse enhances the transmigration of HIV-1 infected monocyte/macrophage [88]. Emerging evidence suggest that HIV-1 virus proteins could prime and activate the NLRP3 inflammasome [89-91]. For this reason, it is worth to investigate whether cocaine could potentiate the HIV-1-induced inflammasome activation. Current evidence indicates that cocaine potentiates the ROS activation in HIV-1-infected macrophage, which is accompanied with the upregulation of inflammasome forming genes [92]. Although the ROS induction was considered as the second activation signal, the direct evidence of increased activity of caspase-1 and processing of IL-1β is still needed. It also suggests NLRP1-caspase-5 axis may contribute to the synergistic effect induced by cocaine and HIV-1 infection. Taken together, current evidences suggest that inflammasome may serve as a converging signaling that mediates the synergistic proinflammatory effect in the macrophage. In future, whether cocaine-induced ER stress could also potentiate the HIV-1-induced inflammasome activation in microglia is worth investigating.

Chronic abuse of Meth is a feature with neurotoxicity marked by diminished dopamine concentration, low level of the dopamine transporter, and neuroinflammation [93-97]. Although with abundant evidence against Meth-induced microglial activation as reviewed above, little study performed on microglia focused on inflammasome. However, the HMGB1 was found to be upregulated in Meth-administered rats and induced the IL-1β production [98]. The author proposed that HMGB1 is released from the Meth-stressed neuron. It has been demonstrated that neuronal-derived HMGB1 is recognized by microglia as the danger-associated signal and primes the NLRP3 inflammasome [99]. As for the second activation signal, there are accumulating evidence suggesting that Meth application induces the mitochondrial damage and ROS production [100-103]. Therefore, in vivo, after priming with neuronal released HMGB1, Meth is very likely to activate the microglia NLRP3 inflammasome through the mitochondrial ROS pathway [104].

**Inflammasome in morphine abuse**

Morphine is originally isolated from poppy straw or opium poppy [105]. It is on the World Health Organization’s list of Essential Medicines as an effective analgesic medicine [106]. Up to now, morphine is still one of the most effective drugs to treat both acute and chronic severe pain. However, the clinical application of morphine is limited by its long-term deleterious effects including addiction and withdrawal symptoms [107-109]. In chronic pain management, the tolerance of morphine will occur on repeated use of the drug, which increases the dose of the drug to achieve the same extent of pain relief [110]. The increased amount of drug, in turn, promotes the development of drug dependence. To prevent the diminished morphine responsiveness in a patient, multiple hypotheses were raised including μ-opioid receptor desensitization, synaptic plasticity changes, and morphine-induced microglial activation and proinflammatory cytokines release [111-115]. In this review, we primarily focus on the role of inflammasome-induced cytokines release in morphine tolerance.

Traditionally, morphine was defined as an immunosuppressive drug that subjects prolonged abuse under susceptibility of various infectious diseases [116, 117]. Although many basic functions of both innate and adaptive immune system such as phagocytic activities of macrophages or T, B-cell antibody response
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were inhibited by morphine, the production of proinflammatory cytokines is, in general, increased by morphine administration [118]. Further studies discovered that morphine promoted the translocation of intestinal bacteria that induces the sepsis in mice [119-121]. Priming with morphine significantly potentiated proinflammatory responses in LPS administrated rats and promoted the progression of sepsis to septic shock [122]. All these evidence provide an alternative explanation for morphine-induced vulnerability to infection, which is achieved by impairment of pathogen clearance capability while augmenting pathogenic inflammatory response. Taken together, the immune modulation of morphine is much more complicated than we thought.

Recently, emerging evidence indicate that while morphine administration systematically dampens the activation of the peripheral immune system, local CNS resident immune cells, microglia are overactivated [123-125]. Because cytokines released from activated microglia might alter the neuronal adaption to morphine [126, 127], the role of neuroinflammation in the development of morphine tolerance was extensively investigated. Further studies confirmed that morphine could non-stereospecifically bind to the accessory protein of TLR4, triggering the TLR4 oligomerization and proinflammation [123]. It has been demonstrated that while μ-opioid receptor expressed on microglia mediated analgesic effect of morphine [124], the microglial TLR4 is critical to the development of morphine tolerance [128, 129]. Inspired by this, following studies targeted microglial activation and subsequent cytokines release and successfully blocked the chronic morphine-induced tolerance [130, 131]. Among those of morphine-induced cytokines, IL-1β stands out for its strong anti-analgesic effects against morphine and important role in morphine tolerance [132, 133]. Administration of morphine significantly stimulate the IL-1β release mediated by TLR4 signaling, and IL-1 receptor antagonization substantially reverses the morphine tolerance [115, 134]. Because NLRP3 activation is primed by TLR4 activation and lead to the release of IL-1β, the NLRP3 inflammasome is receiving more and more attention in research field of morphine tolerance.

After chronic administration of morphine (10 mg/kg, twice a day for 7 days), the western blot analysis of spinal cord indicated an increase of processing of IL-1β, while the pro-IL-1β was not significantly changed [135]. In consistent with this, the activation form of caspase-1 was also significantly elevated while pro-enzyme form remains constant [135]. To confirm the morphine-induced NLRP3 inflammasome activation in vitro, the authors also performed the experiments in BV-2, a cell-line of microglia. After treatment of 200 µM morphine, with or without the additional ATP signal, both the NLRP3 and pro-IL-1β were significantly upregulated. Combined with the second activation signal provided by ATP, they detected a potent processing and release of IL-1β and caspase-1 in supernatant [135]. All results indicate that morphine could work as the first priming signal, which allow microglia response to the second neuronal danger-associated signal (ATP). In addition to this, they also found that morphine induced a strong production of mitochondrial ROS that might work as a second activation signal. After pre-administration of procyanidins, a potent free radical scavenger, both the morphine-induced activation of mitochondrial ROS and NLRP3 inflammasome were blocked [135]. According to this study, it seems that morphine could both prime and activate the NLRP3 inflammasome simultaneously. In contrast to this, a study focused on spinal dorsal horn microglia indicated that morphine only prime the NLRP3 inflammasome through the TLR4-NF-κB pathway, while the second signal was induced by DAMPs released from injured neuron [136]. In their rat model with priming of a short course of morphine, a second prolonged enduration of chronic constriction injury (CCI)-allodynia was found to be mediated by microglial NLRP3 inflammasome. In a combination of morphine and CCI, the level of microRNA-223 that negatively regulated the expression of NLRP3 was significantly decreased. The other essential components of NLRP3 inflammasome were all upregulated (TLR4, NLRP3, pro-Caspae-1, pro-IL-1β). As for the P2X7R, the critical receptor sensing the ATP released from an injured neuron was also elevated. Taken together, morphine induces a persistent sensitization status of microglia that prones to be activated in response to neuronal DAMPs. After the introduction of P2X7R selectively inhibitor, the morphine-induced development of sensiti-
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In summary, in response to a broad range of damage-associated molecular patterns (DAMPs) signals, microglial inflammasome, as an proinflammatory intracellular receptor, can be activated in the course of drug abuse. Systematic and brain stimuli induced by addictive drugs converge signals on microglial inflammasome (Figure 1). Addictive drugs could, on one hand, induce local danger-associated molecular patterns (DAMPs) expression and release from the neuron (HMGB1). On the other hand, the drugs also cause the blood-brain barrier (BBB) damage that allows peripheral cytokines, endotoxins, and HIV-infected monocytes to cross through the BBB. These signals/molecules target the microglia and upregulate the pro-IL-1β and pro-IL-18. The red arrows in Figure 1 indicate the local and systematic stimuli that work as the first priming signal for microglial inflammasome. The addictive drugs could induce potassium efflux (1), ROS activation (2) and ER stress (3) in microglia, which serve as the second activation signals (highlighted in yellow) for microglial inflammasome. Taken together, addictive drugs induce both the first priming and the second activation signals for the activation of microglial inflammasome, which promotes the maturation and release of IL-1β and IL-18. The excessive release of IL-1β produces neuroinflammation, resulting in neuronal damage.

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