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Review:

Regulatory mechanisms and therapeutic potential of microglial inhibitors in neuropathic pain and morphine tolerance*

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Abstract: Microglia are important cells involved in the regulation of neuropathic pain (NPP) and morphine tolerance. Information on their plasticity and polarity has been elucidated after determining their physiological structure, but there is still much to learn about the role of this type of cell in NPP and morphine tolerance. Microglia mediate multiple functions in health and disease by controlling damage in the central nervous system (CNS) and endogenous immune responses to disease. Microglial activation can result in altered opioid system activity, and NPP is characterized by resistance to morphine. Here we investigate the regulatory mechanisms of microglia and review the potential of microglial inhibitors for modulating NPP and morphine tolerance. Targeted inhibition of glial activation is a clinically promising approach to the treatment of NPP and the prevention of morphine tolerance. Finally, we suggest directions for future research on microglial inhibitors.

Key words: Microglia; Neuropathic pain (NPP); Morphine tolerance; Microglial inhibitor
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1 Introduction

Microglia control multiple functions by controlling central nervous system (CNS) damage and endogenous immune responses to disease. In recent years, it has been recognized that microglia have a dual role in traumatic brain and spinal cord injury (SCI): on the one hand, promoting tissue recovery, but on the other hand, causing neurodegeneration. Microglia, which are equivalent to macrophages in the brain and spinal cord, are the first and most important immune defense in the CNS (Ransohoff and el Khoury, 2015). When an acute inflammatory reaction occurs, glial cells activate and then release pro-inflammatory factors (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, interferon (IFN)-γ). The inflammation is dissipated when the glial cells devour toxic substances. Microglia are continuously activated in chronic inflammation, and a large number of pro-inflammatory factors or other neurotoxic substances are released, directly affecting the phagocytic ability of microglia to remove toxic substances. These toxic substances cause different degrees of damage to neurons and produce neurotoxicity (Hickman et al., 2008). The neurobiological properties of microglia are vital aspects of understanding these processes and their corresponding physiological, biochemical, and behavioral consequences (Salter and Beggs, 2014).

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Microglia also express a range of characteristic receptors: chemokine and cytokine receptors, complement factor receptors, and the essential neurotransmitter receptors in the peripheral and CNSs, such as opioid receptors (Yahyavi-Firouz-Abadi et al., 2007), glutamate receptors (Su, 2008), purine receptors (Stokes et al., 2017), neuropeptide receptors (Block et al., 2006), and γ-aminobutyric acid (GABA) receptors (Kuhn et al., 2004) (Fig. 1). Recent studies have shown that the receptor characteristics of microglial activation (Tozaki-Saitoh et al., 2019) play an important role in the development of neuropathic pain (NPP). With the limited understanding of the mechanisms of the origins of NPP, current treatment regimens are not satisfactory. The treatment of NPP with opioids always causes side effects such as morphine tolerance, which can worsen over time (Ochiai et al., 2016). The activation of microglial is closely related to the occurrence of tolerance and dependence. Microglial inhibitors inhibit glial cells by participating in the regulation of multiple signaling pathways in the body and the activation of other glial cells (Bulduk et al., 2019). These mechanisms provide pharmacological opportunities to interfere with NPP and morphine tolerance. In this paper, we report an investigation of the regulatory mechanisms of microglia in NPP and morphine tolerance and review the current state of research on microglial inhibitors. Then, we explore the potential of related microglia inhibitors as therapeutic targets for NPP and morphine tolerance.

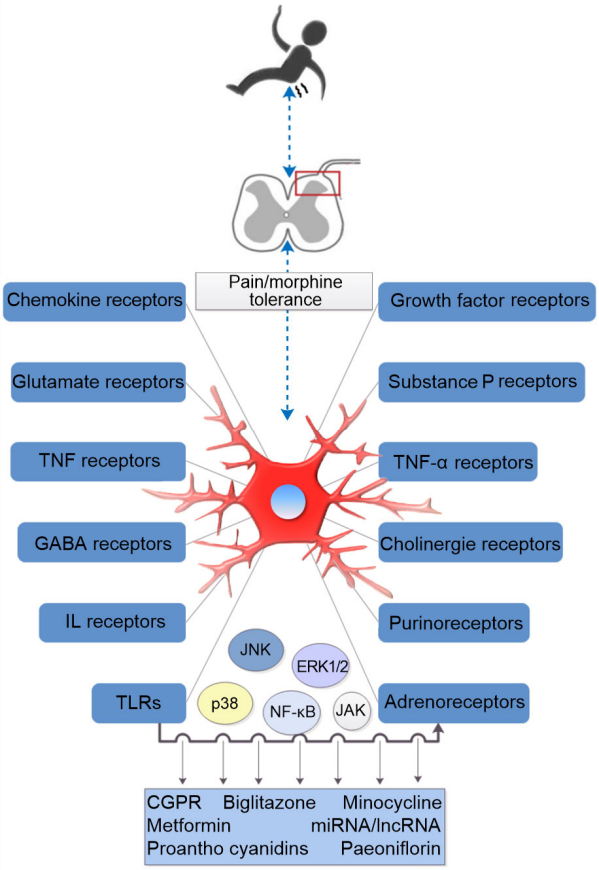

Fig. 1 Roles of microglial inhibitors in the development of NPP and morphine tolerance

Activation of microglial receptors leads to activation of numerous intracellular cascades. Microglial inhibitors influence neuropathic pain (NPP) development and morphine tolerance through signaling pathways. The consequence of activation of these cells is the production of nociceptive and antinociceptive factors that are important for the development of pain and morphine tolerance. Microglial cells express a wide spectrum of neurotransmitter receptors (γ-aminobutyric acid (GABA) receptors, adrenoreceptors, dopamine receptors, and purinoreceptors) and receptors for hormones and modulators (histamine, opioids, substance P, neurotrophins, chemokines, interleukins (ILs), and tumor necrosis factor (TNF)-α). Mitogen-activated protein kinases (MAPKs) are crucial players in cell signaling and transmit a broad range of extracellular signals to mediate various intracellular responses that contribute to the development and maintenance of neuropathy. Inhibition of extracellular signal-regulated protein kinase 1/2 (ERK1/2) activation reduces symptoms of NPP, increases opioid effectiveness, and diminishes pain perception. Inhibition of p38 kinase activation reduces symptoms of NPP, but does not change the expression of antinociceptive factors. Other signaling pathways have similar effects. TLR: Toll-like receptor; JNK: c-Jun N-terminal kinase; NF-κB: nuclear factor κB; JAK: Janus kinase

2 Microglia and NPP

Microglia account for about 10% of the macrophage population in the CNS (Xu et al., 2016; Bulduk et al., 2019). In normal adult brain tissue, the microglia are in a resting state and have small cell bodies with very small protrusions. When stimulated by events such as trauma, infection, physicochemical, or electrical stimulation, they become activated, the cell body enlarges, the protrusions thicken, and the spine becomes clearer. These microglia are called the "early response type." In the presence of stimulation, the cell bodies become further hypertrophied, and the neurites become macrophage-like "phagocytic" microglia (Fan et al., 2018). Microglia plasticity studies focus on the morphological changes of microglia by calculating

the increase in cell size and total cell size (Cogut et al., 2018) and therefore are limited to qualitative analysis at the molecular level. Like other tissue macrophages, microglia exhibit different phenotypes, and have multiple effector functions known as "immune sentinels" when infection occurs (Ginhoux et al., 2013; Bolós et al., 2018). Microglia play an essential role in promoting local innate and adaptive immune responses and maintaining brain homeostasis. They have a dual phenotype (Tang and Le, 2016): the M1-type activates microglia exerting neurotoxic effects by secreting reactive oxygen species (ROS) and pro-inflammatory cytokines, whereas the M2-type activates microglia producing anti-inflammatory cytokines, neurotrophic factors through Toll-like receptors (TLRs), and complement receptors exerting anti-inflammatory and neuroprotective functions (Lee et al., 2019). These reactions identify red flags and initiate immune surveillance and response.

After peripheral nerve injury (PNI), NPP is accompanied by spinal microglial activation (Colburn et al., 1999; Tozaki-Saitoh et al., 2019). When the peripheral nerve tissue is damaged, microglia reaction is rapid, and damaged or activated nociception neurons from the injured central sensory neurons release adenosine-5'-triphosphate (ATP), excitatory amino acid (EAA), and calcitonin gene-related peptide (CGRP) (Bisht et al., 2018). Receptors CX3CR1 (fractalkine) (Zhao et al., 2017), NK1 (SP), P2X4 (ATP), P2X7, etc. activate microglia, thereby promoting pain processes. Peripheral neuronal degeneration of PNI caused by peripheral neuropathy can directly cause the aggregation and activation of microglia in the spinal cord (Inoue, 2017; Tsuda, 2017). A series of signaling cascades that activate p38 mitogen-activated protein kinase (MAPK) phosphorylation in microglia lead to the synthesis and release of intracellular TNF-α, IL-1β, IL-18, brain-derived neurotrophic factor (BDNF), and cyclooxygenase (COX), and to an increase in prostaglandin E2 (PGE2) (Fig. 1) (Tsuda et al., 2017). These neurotransmitters are released outside the cell, re-regulating excitatory and inhibitory synaptic transmission, and ultimately enhancing the transmission of pain information to the brain. In response to neural activity, activated microglia can continuously monitor the environment inside and outside the cell to perform various functions, such as tight controlling or killing pathogens, and eliminating dead or damaged cells. All this information could guide the development of drugs designed to promote beneficial subpopulations and suppress harmful subpopulations of microglia.

3 Glial cell activation and morphine tolerance

It has been reported that long-term use of morphine by patients with NPP leads to morphine tolerance (Ochiai et al., 2016). Opioids are the primary treatment for chronic pain, requiring higher doses of medication over time to affect the same degree of analgesia. Continuous exposure to morphine in the whole body or spinal cord can lead to abnormal pain characterized by thermal hyperalgesia and mechanical allodynia (Ruan et al., 2019). Microglia markers cluster of differentiation molecule 11b (CD11b) and ionized calcium-binding adaptor molecule 1 (Iba1), as well as sputum P2X receptors, are strongly up-regulated in spinal microglia, but are barely expressed in the resting state (Wen et al., 2011; Xu et al., 2014). These changes are consistent with the small colloidal activation observed after neurite damage in the CNS. Glial cell activation and oxidative stress are important factors in inducing opioid side effects (e.g., tolerance and dependence) (Esmaeili-Mahani et al., 2015). CNS glial cells are non-neuronal cells that work with neurons to regulate the homeostasis of the nervous system, mediating and affecting pain. Long-term use of morphine leads to the activation of glial cells. Recent studies have shown that glial cell activation caused by morphine tolerance also occurs at the level of the spinal cord (Jokinen et al., 2018). In studies of spinal nerve ligation (SNL) in rat models, microglia activation contributes to morphine tolerance during maintenance of NPP. The small glial cell inhibitor minocycline delays the development of morphine tolerance, but does not reverse existing morphine tolerance during maintenance of NPP in rats (Zhang et al., 2015). Numerous studies (Esmaeili-Mahani et al., 2015; Takemoto et al., 2016; Zhang et al., 2017; Jokinen et al., 2018) have confirmed that glial cells are involved in morphine tolerance, but the location of glial cells associated with morphine tolerance is still controversial. We hypothesize that inhibition of spinal glial activation can attenuate morphine tolerance and thereby improve the therapeutic effect of morphine.

3.1 Microglial activation: a bridge between NPP and morphine tolerance

Among the published evidence-based recommendations for NPP treatment, the first-line drugs include antidepressants, anticonvulsants, topical and opioid (μ-opioid peptide (MOP), κ-opioid peptide (KOP), non-opioid peptide (NOP)) analgesics (Zilliox, 2017). The tolerance resulting from the application of opioid morphine has become a problematic aspect in the treatment of NPP (Edwards et al., 2016; Widerström-Noga, 2017). NPP is relatively less responsive to opioids than other types of pain. This might be due to the microglial activation caused by severe neuroinflammation, leading to the destruction of the opioid system, which can inhibit the effect of morphine and other opioid drugs on pain control (Popiolek-Barczyk et al., 2017). Microglia communicate with neurons, astrocytes, and other cells, including cells of the immune system, through several receptors and signaling pathways. Activated glial cells synthesize and release large amounts of glial mediators such as cytokines, chemokines, growth factors, and proteases, promoting interactions between glial cells and glial cell-neurons (Popiolek-Barczyk and Mika, 2016; Popiolek-Barczyk et al., 2017). Many studies have attempted to determine the roles of these intracellular pathways, which might be involved in the development and maintenance of NPP and morphine tolerance.

The pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and chemokines (CC motif ligand 2 (CCL2) and CXC motif ligand 1 (CXCL1)) are widely studied colloidal media (Mika, 2008). Both pro-inflammatory and anti-inflammatory cytokines are involved in the formation and maintenance of morphine tolerance. There is growing evidence that cytokines play a crucial role in inducing over-activity or central sensitization in the dorsal horn neurons. Blocking these cytokines has been shown to reduce NPP and morphine tolerance (Feng et al., 2012; Sun et al., 2012). For patients with long-term use of morphine, pre-application of microglial inhibitors can be used as a clinical pain adjuvant therapy. The use of microglial inhibitors can reverse the expression of pro-inflammatory factors during morphine infusion. The expression of microglial surface markers Iba-1, purinergic receptors (P2X4R and P2X7R), and TLR4 increases with increasing levels of glial medium (Tsai et al., 2016; Bisht et al., 2018). Targeting microglial activation may have great research value for the treatment of NPP and the prevention of morphine tolerance.

3.2 Insights into microglial signaling pathways for NPP and morphine tolerance

MAPK is a group of serine-threonine protein kinases activated by different extracellular stimuli. It regulates various physiological activities such as neuronal plasticity, neuronal apoptosis, and inflammatory response. MAPK is divided into four subfamilies, p38, c-Jun N-terminal kinase (JNK), extracellular signal-regulated protein kinase 1/2 (ERK1/2), and ERK5, which are expressed in different cell types and form different MAPK cascade pathways. Among them, p38 is expressed primarily in the microglia. Prolonged use of morphine induces glial p38 MAPK activation, and initiates a series of inducible nitric oxide synthase (iNOS) expression, leading to NO-mediated spinal neuron degeneration (Guo and Bhat, 2006; Xu et al., 2006). At the same time, it can regulate spinal synaptic transmission, induce excitability of nociceptive neurons in the spinal dorsal horn, and lead to morphine tolerance by modulating downstream signaling molecules such as transient receptor potential vanilloid 1 (TRPV1), IL-1β, IL-6, and TNF-α (Cai et al., 2016). Microglial inhibitors have been reported to reduce spinal cord levels in the context of NPP (Bulduk et al., 2019). These actions are associated with the inhibition of microglial activation and reduce the symptoms associated with neuropathy. p38 kinase is also closely associated with nuclear factor κB (NF-κB) signaling in cultured microglia (Liu et al., 2019; Youssef et al., 2019). Another member of the MAPK family, ERK1/2, is also activated in glial cells during NPP (Yu et al., 2016). An increased release leads to morphine tolerance and induces analgesia. Targeting CGRP-related signaling molecules can prolong or restore the analgesic properties of morphine after prolonged exposure. In cultured glial cells, morphine increases phosphorylated ERK (pERK) expression by modifying the Akt pathway upstream of ERK1/2 and iNOS (Berta et al., 2013; Merighi et al., 2013). These data suggest that regulation of microglial signaling can provide a means to attenuate glial cell activation, thereby interfering with the development of tolerance to, and dependence on, opioids.

4 Development of NPP and morphine tolerance due to the influence of microglial inhibitors

Studies have shown that mice with NPP exhibit morphine tolerance due to a decrease of morphine concentration in the brain. Ossipov et al. (2003) suggested that opioid-induced hyperalgesia is a contributing factor to opioid anti-receptive tolerance in patients with NPP. Microglia with altered morphological function produce inflammatory mediators such as IL-1β, IL-6, and TNF-α in one activation pattern similar to NPP. They also induce excessive excitation of dorsal horn nociceptive neurons in the spinal cord (Jokinen et al., 2018), mediate the glial–neuron cascade reaction, activate a series of anti-opioid-like neuropeptides (Cai et al., 2016), exacerbate the progression of pain, and promote morphine tolerance. Microglia inhibition was found to be mediated by chemokines, not CCL2. The aforementioned inflammatory factors have been shown to have strong inhibitory effects on glial cell activation and CCL7 release. Similarly, lowering spinal CCL7 levels can also inhibit microglial activation and NPP (Li J et al., 2017; Xie et al., 2018). Intrathecal injection of CCL2 and CCL7 neutralizing antibodies not only attenuates chronic pain-induced injury of the sciatic nerve (chronic constriction injury (CCI)), but also enhances morphine-induced analgesia (Kwiatkowski et al., 2019). Thus, inhibition of glial cell activation and its interaction with neurons can reduce NPP and delay morphine tolerance. It has been demonstrated that minocycline, as a microglial inhibitor, can prolong the effect of morphine under NPP conditions (Mika et al., 2007). Other studies have shown that some natural drugs, partial hypoglycemic drugs, CGRP antagonist, microRNAs (miRNAs), purine receptor inhibitors, and synthetics can also affect NPP and morphine tolerance by inhibiting the activation of glial cells.

4.1 Minocycline

Minocycline is a second-generation drug from the tetracycline family of antibiotics, and is usually considered as a "microglial inhibitor" or "inhibitor of neuroinflammation" (Bulduk et al., 2019). Studies have shown that minocycline affects microglia by inhibiting the activation of intracellular signaling pathways and the release of pro-inflammatory factors (Piotrowska et al., 2017). These intracellular signaling pathways are the physiological basis for the development of microglia-mediated NPP and morphine tolerance. The role of minocycline as a microglial inhibitor in the formation of NPP and morphine tolerance has been extensively studied. For example, Moini-Zanjani et al. (2016) found that minocycline reduced IL-6 concentration in microglia in a rat model of CCI with NPP. Cui et al. (2008) suggested that intrathecal injection of minocycline significantly inhibits the increase of p38 MAPK activation in spinal microglia induced by chronic intrathecal morphine, demonstrating that minocycline can antagonize morphine analgesic tolerance. Also, minocycline preconditioning reduces the growth of microglial cells at the site of glioma-induced injury in rodent models of SCI. More importantly, minocycline can also prevent the adverse effects of morphine on the recovery of motor and sensory functions without destroying its analgesic effect (Aceves et al., 2019). Zhang et al. (2019) found that in a rat model of SNL, microglial activation contributed to morphine tolerance during NPP maintenance. Minocycline delayed the development of morphine tolerance, but did not reverse the existing morphine tolerance during the maintenance of NPP. These findings may be useful for clinical pain management. A clinical trial of patients with lumbar radicular pain found that on the 14th day of treatment, minocycline was less effective than placebo based on a digital pain rating scale, casting doubt on whether the effect of minocycline is clinically significant. It may be that the sample also has some other influencing factors (Vanelderen et al., 2015). Another clinical trial of patients with NPP found that minocycline failed to reduce pain intensity after four weeks of treatment, but successfully reduced the emotional pain associated with NPP (Sumitani et al., 2016). The optimal timing and duration of clinical application are still unclear, and the clinical effect of minocycline on NPP remains an open research space.

4.2 Natural medicines and their extracts

Paeoniflorin is a biologically active monoterpene glucoside with anti-inflammatory and antioxidative effects (Li H et al., 2017). It inhibits the expression of IL-1β, IL-6, and TNF-α by inhibiting the phosphorylation of the upstream protein apoptosis

signal-regulating kinase 1 (ASK1) of p38 and JNK, thereby delaying and alleviating the progression of NPP (Zhou et al., 2019). Studies have shown that paeoniflorin inhibits phosphorylation of p38 MAPK, NF-κB translocation, and pro-inflammatory cytokine expression by inhibiting morphine-induced activation of glial cells, leading to an increase in acute analgesia of morphine and a decrease in morphine chronic antinociceptive tolerance (Jiang et al., 2015). Resveratrol, a natural non-flavonoid polyphenolic compound, has multiple protective effects in the CNS (Bobermin et al., 2018). In tactile allodynia caused by SNL, intrathecal injection of resveratrol can reduce the activity and expression of NOS, and alleviate tactile allodynia in rats with NPP (Pérez-Severiano et al., 2008). In a study by Tsai et al. (2016), intrathecal injection of resveratrol reversed the increase in histone deacetylase 1 (HDAC1), TNF-α, and TNF receptor 1 (TNFR1) expression induced by long-term morphine infusion, and restored the antinociceptive effect in morphine-tolerant rats. Proanthocyanidins (PAs) are polyphenols in plant foods that have many health benefits, including cancer prevention, cardiovascular protection, and diabetes prevention (Zhang et al., 2016). PAs combined with morphine inhibit the morphine-induced increase in IL-1β and the activation of nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome, decrease the activation of p38 MAPK, inhibit the level of ROS in microglia, and attenuate the development of acute and chronic morphine tolerance (Cai et al., 2016). Curcumin (tetracosylmethane), a component of turmeric or curry powder, is a natural polyphenol product isolated from the rhizome of the plant turmeric (*Curcuma longa*) (Aggarwal and Sung, 2009). Curcumin can inhibit the inhibition of Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα) activity in the CNS. CaMKIIα is often expressed in the spinal dorsal horn and dorsal root ganglia simultaneously with the MOP in the surface layer. Activated CaMKII has been shown to phosphorylate *N*-methyl-D-aspartate (NMDA) receptors, which in turn mediate the desensitization of MOPs (Hu et al., 2015). Other studies have shown that curcumin has some anti-inflammatory and analgesic effects on individuals with NPP. Curcumin can be regarded as an effective adjuvant therapy, which can be combined with other drugs used in conventional treatment, thereby alleviating NPP (di Pierro and Settembre, 2013). In other studies, natural drugs such as ligustrazine, baicalin, and Yokukansan have also shown the corresponding effects of inhibiting glial activation and delaying morphine tolerance (Takemoto et al., 2016).

4.3 Calcitonin gene-related peptide antagonist

Chronic morphine induces the synthesis of CGRP, which acts on CGRP receptors located on astrocytes and microglia (Wang et al., 2009). CGRP regulates the activation of the neuronal CaMKII–cyclic adenosine monophosphate (cAMP)-responsive element-binding protein (CREB), microglia p38–NF-κB, and astroglial ERK–signal transducer and activator of transcription 1 and 3 (Stat1/3) cascades, contributing to increased synthesis and release of pro-inflammatory mediators (He et al., 2017), and promoting the development of morphine-induced analgesic tolerance (Wang et al., 2010a). Intrathecal treatment with the non-peptide CGRP receptor antagonist (BIBN4096BS) blocks behavioral and morphological changes such as microglial hypertrophy, and increases the number of branches. This result provides morphological evidence for the role of CGRP in the development of morphine antinociceptive tolerance (Wang et al., 2010b). The pathological mechanism of clinical migraine shows that the release of CGRP in the caudate nucleus of the trigeminal nerve can promote the activation of second-order nociceptive neurons and glial cells, suggesting that CGRP is involved in the development and maintenance of persistent pain, central sensitization, and allodynia. The use of the clinical olcegepant (BIBN4096BS) and telcagepant (MK-0974) is effective in the acute treatment of migraine, demonstrating the potential of CGRP receptor antagonists for the treatment of other NPP (Crowley et al., 2015). CGRP monoclonal antibodies have excellent safety and tolerability in episodic and chronic migraines (Mitsikostas and Reuter, 2017).

Adrenomedullin (AM) belongs to CGRP family and has been recently demonstrated to be a pain-related peptide. It has also been shown that the expression and release of AM are increased in the dorsal root ganglion (DRG) and spinal dorsal horn during inflammation and repeated use of morphine, suggesting that enhanced AM receptor signaling in the DRG and spinal dorsal horn contributes to the induction of

inflammatory pain and morphine tolerance (Zeng et al., 2014; Huang and Hong, 2015; Chen et al., 2017). These receptors are called AM1 and AM2. The AM1 receptor is highly selective for AM relative to CGRP and other peptides, and AM_{22-52} is a potent antagonist of this receptor. The AM2 receptor has lower specificity for AM and a significant affinity for βCGRP. Whenever appropriate, it appears that βCGRP might activate the CGRP1 and AM2 receptors, and AM can activate the AM1 and AM2 receptors as well as the CGRP1 receptor. Current peptide antagonists are not selective for these three receptors (Hay et al., 2004). Long-term exposure to morphine increases AM receptor signaling, spinal cord AM bioactivity, expression of neuronal NOS (nNOS) in the spinal dorsal horn and DRG neurons, which in turn promotes the development and maintenance of morphine tolerance (Wang et al., 2014). AM biological activity is positively correlated with CCL2 expression in the DRG (Chen et al., 2017). Intrathecal administration of AM_{22-52} also abolishes bone cancer-induced pain hypersensitivity and the increase in *CCL2* mRNA levels (Chen et al., 2017), which supports the role of CGRP antagonists in relieving NPP and morphine tolerance.

4.4 Partial hypoglycemic drugs

Diabetic NPP is one of the most common and debilitating complications of diabetes, and available treatments are not effective (Redivo et al., 2019). In a type 1 diabetic streptozotocin (STZ)-diabetic rat model, compared with a fully developed diabetic group, the development of both mechanical allodynia and opioid hyposensitivity was prevented in a group that used insulin implants to restore and maintain normal blood glucose levels (Otto et al., 2011). The results showed that the effective control of hyperglycemia could reduce the abnormal pain of animals and improve the sensitivity of opioids. Therefore, many hypoglycemic agents could affect the progress of NPP and the development of morphine tolerance by affecting the activation of microglia. It also has been found that morphine induces the release of heat shock protein 70 (HSP70), which can activate microglial cells, and trigger TLR4 to mediate inflammation, leading to p38 proliferation of MAPK, NF-κB, p65, and NLRP3 inflammasome. Glibenclamide is a clinical hypoglycemic agent, which can significantly inhibit the release of morphine-induced HSP70 and the neuroinflammation mediated by the inflammation of HSP70-TLR4-NLRP3, thus reducing the tolerance of morphine (Qu et al., 2017). Pioglitazone is a thiazolidinedione antidiabetic drug, an insulin sensitizer, and a peroxisome proliferator-activated receptor γ (PPAR-γ) agonist. Intraperitoneal injection of pioglitazone significantly reduces the up-regulation of dorsal horn CD11b, glial fibrillary acidic protein (GFAP), and phosphorylated p38 (p-p38) induced by nerve injury, implying a mechanism of action involving the activation of spinal microglia and/or astrocytes. PPAR-γ activation can reduce or prevent the development of established NPP (Morgenweck et al., 2013). The inhibition of glial cell activation and pro-inflammatory responses are also a possible mechanism by which pioglitazone is delayed and attenuated by morphine tolerance (Ghavimi et al., 2014). Co-administration of pioglitazone with morphine not only reduces morphine-induced tolerance, but also blocks the up-regulation of pro-inflammatory cytokines (Koh et al., 2018), NFs, as well as the activity of κB in the rat cerebral cortex (Ghavimi et al., 2015). Metformin is a biguanide antidiabetic drug and an AMP-activated protein kinase (AMPK) activator with potential anti-inflammatory effects. After morphine activates glial cells, the up-regulation of p38 MAPK phosphorylation, a pro-inflammatory cytokine, and *TLR-4* mRNA expression can be inhibited by metformin. Systemic injection of metformin can significantly block the activation of morphine-induced spinal microglia, thereby weakening the development of chronic morphine tolerance in mice (Pan et al., 2016). Metformin is effective in reversing neurological hypersensitivity reactions that are associated with a reduction in Iba-1 staining in the dorsal horn of microglial activation markers. In a retrospective study of metformin in the treatment of lumbar radicular pain, after comparing the pain outcomes of 46 patients treated with metformin with those of 94 patients who were not, Taylor et al. (2013) found that the use of metformin reduced lumbar radicular pain. Larger retrospective studies are needed to distinguish whether metformin acts directly as an analgesic or as an anti-allergic drug in the context of chronic NPP (Taylor et al., 2013). A case report also provided evidence that metformin provides sufficient pain control (Labuzek

et al., 2012). These findings demonstrate the possible application of hypoglycemic drugs in clinical pain management.

4.5 miRNAs

miRNAs are small non-coding functional RNAs that regulate target gene expression by binding to the 3'-untranslated region (3'-UTR) of mRNA in a Dicer-dependent manner (Chen et al., 2020). Some miRNAs are capable of regulating intracellular μ-opioid receptor (MOR) biosynthesis as a negative feedback regulator (Wu et al., 2013). MOR agonists such as morphine and fentanyl also regulate miRNA expression (Zheng et al., 2010). Modulation of miRNAs also prevents opioid-induced microglial damage (Qiu et al., 2015). Dysregulation of miRNA plays an important role in the formation and maintenance of NPP. miRNAs in activated glial cells induce the formation of NPP microglia which regulate neurotransmission and neuroinflammation, adding to evidence that miRNAs may be the primary participants in NPP (Ji et al., 2013).

miRNA-21 (miR-21) expression in damaged DRG neurons continues to be up-regulated after pain progression, but not in adjacent intact DRG neurons. Intrathecal administration of IL-1β also increases miR-21 expression in DRG neurons. Furthermore, mechanical allodynia and thermal hyperalgesia in NPP are attenuated by intrathecal administration of miR-21 inhibitors (Sakai and Suzuki, 2013). There is evidence that miRNA expression might be the foundation for neurological adaptation that mediates tolerance to morphine analgesia (Tapocik et al., 2016). miR-873a-5p, miR-219-5p, and miR-365 (Wang et al., 2016, 2017; Weng et al., 2019) reduce the expression of NMDA receptor subunit 1 (NR1) by inhibiting the CaMKII/NMDA receptor pathway, resulting in a decrease in morphine tolerance. miR-365 reduces the levels of IL-1β, TNF-α, and IL-18 by targeting β-arrestin 2 and reduces the analgesic tolerance of morphine by inhibiting the activation of the ERK/CREB signaling pathway (Wu et al., 2018). Also, overexpression of miR-365 can reverse an established morphine tolerance (Wang et al., 2016). miR-223 down-regulates NLRP3 to inhibit the activity of NLRP3 inflammatory bodies (NLRP3, apoptosis-associated spot-like protein, and caspase-1), alleviating morphine tolerance in rats (Xie et al., 2017). Although many miRNAs are abnormally expressed in chronic pain and morphine tolerance, few miRNAs overlap. Dai et al. (2018) found that only one miRNA (miR-124) was deregulated in microglia in NPP and morphine tolerance. Therefore, miR-124-based treatment is an important potential treatment. Some scientists have suggested that miR-124 has multiple targets in the brain at the same time. It is known that multiple factors usually induce CNS diseases; therefore, alternative therapy with miR-124 may be more advantageous in the treatment of multi-factor CNS diseases, and deserves further research (Guo et al., 2019).

5 Prospects for future microglial inhibitors

Promoting microglial inhibitors from animal research to clinical applications remains challenging. Consequently, it is crucial to test potential compounds that can be used in human therapy in basic research experiments. The intracellular pathways involved are the basis of many physiological functions, and their non-selective and complete inhibition could lead to side effects. Many microglial inhibitors have been characterized in studies with animal models and in vitro, and several have now entered clinical trials (Harris et al., 2016). With the exception of some of those described above, studies of microglial inhibitors have been significantly hampered by a lack of selective microglial inhibitors. For a long time, researchers have relied on non-selective P2X receptor antagonists such as Brilliant Blue G (BBG), $P2Y_{12}$ antagonist MRS2395, and clopidogrel (adenosine diphosphate (ADP) $P2Y_{12}$ receptor antagonist) to research microglial inhibitors. However, these agents have a low affinity for microglia and are more active on other targets. Recent evidence suggests that the importance of microglial inhibitors has raised interest in the development of selective receptor antagonists. The discovery of selective microglial inhibitors will provide a new way for scientific research to target NPP and morphine tolerance selectively. Table 1 lists some microglial inhibitors that have been studied to date. Most researchers in the field are proceeding cautiously, testing their results on mice, rats, and humans, with some success. However, this area can become overreaching.

Table 1 Microglial inhibitors

Category	Compound	Potency at IC_{50} (species)	Reference
Toll-like receptor	Girollion	77–215 nmol/L (*Plasmodium falciparum*)	Benoit-Vical et al., 2008
	Dexmedetomidine	(170±20) μmol/L (human)	Stoetzer et al., 2016
	Propofol	4.97 μg/mL (human)	Hannam et al., 2016
p38 kinase inhibitor	PD169316	89 nmol/L (mouse)	Davies et al., 2000
	SB203580	1 mmol/L (mouse)	Davies et al., 2000
	SB202190	50 nmol/L (mouse)	Davies et al., 2000
	SB202190	100 nmol/L (mouse)	Davies et al., 2000
Inhibitors of the PI3K/Akt pathway	Wortmannin	30 nmol/L (human)	Blommaart et al., 1997
RIPI inhibitor	7-Cl-*O*-Nec-1 (Nec-1s)	Not determined	Not reported
	GSK963	1–4 nmol/L (human and murine)	Berger et al., 2015
RAGE inhibitor	FPS-ZM1	(25±5) nmol/L (hamster)	Deane et al., 2012
	Aminopyridine	50.49 mmol/L (human)	Ghorab et al., 2014
	Thiazole compound	Not determined	Not reported
Histone deacetylase inhibitor	Pracinostat	40–140 nmol/L (human)	Lin et al., 2015
	RSV	(23.2±1.3) μmol/L (rat)	Ciddi and Dodda, 2014
PPF	HWA 285	6 mmol/L (rat)	Parkinson et al., 1993
FC	Pentaric acid	(47±6) mmol/L (rat)	Scandroglio et al., 2014

IC_{50}: half maximal inhibitory concentration; PI3K: phosphatidylinositol 3-kinase; RIPI: reactive inhibition/proactive inhibition; RAGE: receptor for advanced glycation end products; PPF: propentofylline; FC: fluorocitrate; RSV: resveratrol

6 Conclusions

Microglial inhibitors appear to be an attractive antinociceptive option due to their ability to block pro-inflammatory cytokine signaling. The regulation of microglial activation by altering the intracellular pathway changes the expression of anti-inflammatory factors, thereby affecting the progression of NPP and morphine tolerance. This approach could provide a basis for the development of compounds with improved therapeutic efficacy in NPP pathology and morphine tolerance. Future research should pay more attention to the role of microglia in the transition mechanisms of pathway-activated polarization.

Contributors

Er-rong DU, Rong-ping FAN, Li-lou RONG, and Zhen XIE wrote and edited the manuscript. Chang-shui XU contributed the study design of the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Er-rong DU, Rong-ping FAN, Li-lou RONG, Zhen XIE and Chang-shui XU declare that they have no conflict of interest.

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中文概要

题　目：小胶质细胞抑制剂在神经性疼痛和吗啡耐受中的调节机制和治疗潜力

概　要：小胶质细胞是参与调节神经性疼痛（NPP）和吗啡耐受性的重要细胞。在确定它们的生理结构后，已经阐明了有关其可塑性和极性的信息，但关于这种类型的细胞在 NPP 和吗啡耐受性中的作用，仍有许多知识要学习。小胶质细胞通过控制中枢神经系统的损伤和对疾病的内源性免疫反应，介导健康和疾病的多种功能。小胶质细胞活化可导致阿片样物质系统活性改变，而 NPP 的特征在于对吗啡的抗性。在这里，我们研究小胶质细胞的调节机制，并综述了小胶质细胞抑制剂抑制 NPP 和吗啡耐受性的潜力。靶向的神经胶质活化是治疗 NPP 和预防吗啡耐受的临床有前途的方法。最后，我们为小胶质细胞抑制剂的未来研究提出了建议。

关键词：小胶质细胞；神经性疼痛；吗啡耐受；小胶质细胞抑制剂