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Review Article

Spinal microglia: A potential target in the treatment of chronic visceral pain

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Abstract

Chronic visceral pain is the predominant symptom of functional gastrointestinal disorders and chronic pancreatitis. Such pain can impair the patients' quality of life, and can also serve as one of the principal reasons for these patients to seek medical help. Nevertheless, the underlying mechanisms of chronic visceral pain have remained unclear, and much of what we know about visceral pain has been derived from studies of somatic nociception. Current treatment of chronic visceral pain has continued to be unsatisfactory, because of unclear pathophysiology. However, recent progress in pain research has identified the important role of spinal microglia in the development of somatic nociception. For visceral pain, several animal studies have demonstrated that spinal cord microglia is activated during the development of visceral hyperalgesia, which can be induced by neonatal colorectal irritation, psychological stress, and trinitrobenzene sulfonic acid-induced pancreatitis. This visceral hyperalgesia is also associated with elevated phosphorylation of p38 mitogen-activated protein kinase. Minocycline (a microglia inhibitor) reversed the hyperalgesia in rat models of chronic visceral pain, whereas fractalkine (FKN, a microglia activator) reproduced the visceral nociception in naïve rats. These preliminary results support the pronociceptive role of spinal microglia in mediating visceral hyperalgesia. Consequently, spinal microglia may serve as a promising target for controlling the chronic visceral pain.

Keywords: microglia; spinal cord; visceral pain

1. Brief of visceral pain

Visceral pain is one of the most common forms of pain produced by diseases arising from the bowel or internal organs. Additionally, visceral pain is one of the most frequent complaints that provokes patients to seek medical help.[1] Notwithstanding these facts, much of the current knowledge about the visceral pain mechanisms of pain is mainly derived from experimental studies in somatic nociception.[2,3] Although the pain processes between visceral and somatic nociception have much in common, they also have several important differences.[3] First, visceral pain is less localized, and is more unpleasant than perceived intensity-matched somatic pain. Second, visceral pain can be manifested in other locations and is usually accompanied by motor and autonomic reflexes, such as nausea, vomiting, and lower-back muscle tension that typically occurs in renal colic.[4] Furthermore, visceral pain cannot be evoked from all viscera. Organs such as the liver, kidney, most solid viscera, and lung parenchyma are not sensitive to pain.[3]

Chronic visceral pain is the predominant symptom of functional gastrointestinal disorder (FGID).[5] FGID accounts for more than half of the patient traffic in GI clinics. Despite extensive research in this area, the pathophysiology of the origin of visceral pain in patients with FGID remains uncertain, a fact which contributes to unfavorable treatment outcomes. The lack of an identified biomarker and firm diagnosis in FGID also results in the increased cost of medical care, loss of work productivity, and impairment of quality of life (QoL) in FGID patients. The combination of extensive investigation and frequent hospital attendance exerts a

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considerable financial burden to society in terms of healthcare resources, and impacts economies in both Western and Eastern countries.[6−8] Another common form of chronic visceral pain is the pain from chronic pancreatitis (CP). Chronic epigastric pain is the cardinal feature of CP, which is usually recurrent, intense, and long-lasting. The pain in CP can lead to malnutrition, narcotic addiction, and major socio-economic problems. However, the control of pain in CP remains a difficult and frustrating challenge for clinicians.[9] Similar to the situation in FGID, the underlying mechanisms by which pain in CP is produced are still unclear.

The pathological pain seen in FGID or CP is usually accompanied by visceral hyperalgesia and allodynia, resulting in a heightened response to noxious or even non-noxious stimuli. The abnormal nociceptive responses can be induced by the neuroplastic changes in primary afferent fibers (peripheral sensitization), as well as in the spinal cord and brain (central sensitization).[5,10] Recent research has demonstrated that persistent activation of spinal microglia may contribute to chronic pain after peripheral and central nervous system (CNS) injury; these findings suggest that treatment by targeting microglia may attenuate or alleviate chronic pain syndrome.[11,12] However, most reports on the contribution of microglia to pain focus on somatic rather than visceral forms of chronic pain. Thus, we will present a brief review starting at the current understanding of the roles of microglia cells in chronic somatic pain, and then in visceral pain.

2. Overview of glia cells

Glial cells, including microglia, astrocytes, and oligodendrocytes in the CNS, have all been considered as the "third element" that simply fills spaces around neurons, the "truly" functional cells of the nervous system.[13] It is widely accepted that glia maintain general homeostasis of the CNS in ways that impact on neuronal function.[12,14] Oligodendrocytes are responsible for myelin formation in axons. Astrocytes, neuroectoderm in origin and contributing to around 40−50% of all glia cells, are thought to play roles in the regulation of potassium, glutamate, and other neurotransmitters in the CNS. Astrocytes would also be activated upon various stimuli and in response to the many byproducts from activated microglia. For microglia, it originates from bone-marrow derived monocytes, and shares a common mesodermal lineage with macrophages. Microglia constitutes about 5−12% of the total glial cell population and is traditionally viewed as the phagocytes in the CNS. Under normal conditions, microglia is in a quiescent status and can be activated or mobilized upon injury, infection, or other diseases states in the CNS.

3. Spinal microglia in chronic neuropathic or inflammatory pain

Traditionally, plastic changes in neurons are thought to play a major role in the sensitization of nociceptive pathways in both chronic somatic and visceral pain. In recent years, the link between microglia and pathological pain states has become more widely acknowledged.[11,13,15] Microglia activation has been found to play a key role in the initiation and/or maintenance of hyperalgesia and allodynia in animal models of chronic neuropathic or inflammatory pain.[12,14,16−19]

Microglia can display significant morphology changes (from ramified to ameboid) in a chronic pain model after peripheral nerve injury (such as nerve transaction or ligation of the sciatic nerve), peripheral inflammation (intra-articular injection of complete Freund's adjuvant), or damage to the CNS (spinal cord injury and spinal infection with human immunodeficiency virus).[20−28] Persistent hyperalgesia and allodynia, as well as release of many pro-inflammatory mediators, can be observed in association with morphological changes of the spinal microglia. Multiple mechanisms may be involved in the activation of spinal microglia during somatic pain processing. Several pathways with associated molecules are reported to be involved in the somatic nociception, such as: (1) chemokine X3C-1 receptor [CX3CR-1, with fractalkine (FKN) as ligand]; (2) interferon-gamma receptor (IFN-gammaR); (3) toll-like receptor; and (4) purinergic receptor.[12] The molecules mentioned have been administered to rodent models and shown to alter chronic pain behaviors. For example, spinal nerve injury will induce an up-regulation of CX3CR1 in spinal microglia.[29] Following peripheral nerve injury, FKN has been found to be released from primary afferents and then bind to its CX3CR1, expressed predominantly in spinal cord microglia, to induce microglial activation.[11,30,31] Thus, FKN can act as a signal between neuron and microglia to induce microglia activation. For IFN-gammaR, rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist showing an ability to down-regulate IFN-gamma-induced gene expression and subsequent reduction of the chemotactic response to chemokine ligand 2, can attenuate the tactile allodynia in a neuropathic pain model.[32] Toll-like receptors (TLRs) are a family which includes 13 receptors that play a key role in the innate immune system to defend the host against pathogens. TLR2 has been shown to play a critical role in morphine-induced microglia activation and dependence.[14] Spinal TLR4 mRNA expression in microglia was increased after L5 nerve transaction in rats, a model of neuropathic pain.[33] And TLR-4 knockout mice developed less neuropathic pain, as well as strongly decreased expression of pain-related cytokines.[34] ATP and its purinergic receptors (P2) are well-known pain mediators.[35] Spinal up-regulation of P2X4 in microglia has been demonstrated to be crucial for tactile allodynia after peripheral nerve injury.[36] Furthermore, pharmacological inhibition or genetic deletion of either P2X4 or P2Y12 alleviates allodynia or heat hyperalgesia after peripheral nerve injury.[37,38] In addition to these major pathways in mediating microglia activation in a chronic somatic pain model, other molecules may also be involved in the somatic nociception. These molecules include MHC class II proteins, glucocorticoid receptors, and cannabinoid type 1 or 2 receptors.[11,12,15]

Once microglia is activated, the activated microglia would increase synthesis and secretion of various cytokines and chemokines, including IL-1β, IL-6, IL-10, tumor necrosis factor (TNF), prostaglandin E2 (PGE2), nitric oxide, and

transforming growth factor-β (TGF-β).[11–14,39] These microglia-released cytokines will also amplify microglia activation themselves in an autocrine manner.[12] In addition, the cytokines may also directly modulate dorsal horn neuron activity involved in the development of pain hypersensitivity. Evidence has also shown that intrathecal injection of cytokines, such as IL-1β, IL6, or TNF-α, can lead to symptoms of neuropathic pain in naïve rats.[40] Furthermore, microglia activation may partially contribute to the induction of the ATP receptors (P2X4, P2X7), p38 phosphorylation, and IL-1 release.[41,42] The above cross-talk between neurons and microglia would play a role in mediating pain behavior sensitization. Besides the cross-talk between microglia and neurons in the pathogenesis of somatic nociception, it has been suggested that astrocytes may also play a role in the initiation and maintenance of chronic pain.[12] Activated astrocytes can also release cytokines and chemokines to enhance and prolong the nociceptive responses.

The intracellular signaling pathways mediating spinal microglia activation in response to cytokines have also been explored. Increased p38 phosphorylation in spinal microglia (activated MAPK pathway) has been demonstrated in an animal model of pathological pain from peripheral inflammation, nerve injury, and spinal cord injury.[8] Pharmacological inhibition of p38 activity can attenuate the development of allodynia and hyperalgesia in various somatic pain models.[20,26,43] Besides p38 activation, extracellular signal-regulated kinase and the nuclear transcription factor NF-κB have been described as the important mediators involved in the intracellular signaling pathways and transcription regulation of the genes encoding for proinflammatory cytokines in spinal microglia after nerve injury.[44,45] This phenomenon provides positive feedback loops in the crosstalk between microglia and neurons, leading to more production of the inflammatory mediators and sequent central sensitization.

The above evidence suggests that microglia plays a crucial role in the development of somatic pain. Thus, immunosuppressive compounds are then developed to attenuate microglial activation, and proved to be effective in relieving neuropathic pain in animal models. For example, minocycline can selectively inhibit microglia, suppress the release of proinflammatory cytokines and p38 phosphorylation, and ultimately be effective in attenuating the neuropathic pain in animal models.[46–49] Despite the positive effect of minocycline in the treatment of ongoing pain, controversial results exist. For example, in the L5 spinal nerve transection model, the initiation of minocycline treatment 5 days after transection does not attenuate allodynia and hyperalgesia, although it does inhibit microglial activation.[50] Similarly, systemic administration of minocycline has no effect on phase 2 formalin-induced flinching or carrageenan-induced hyperalgesia.[46] Interestingly, intrathecal minocycline did reduce the peripheral inflammation-induced hyperalgesia.[46] Minocycline is also not effective in reversing allodynia after sciatic nerve inflammation if the drug is given 1 week after onset, even though minocycline did show some effect at the initial phase of allodynia.[47] The reason for these widely different results is still unclear and further studies are necessary to better explain the contradictory results.

4. Spinal microglia in chronic visceral pain

Although visceral pain is an important component of the normal sensory function of a human being, surprisingly, the study of the role of spinal microglia in chronic visceral pain is extremely rare and has only recently been addressed. Saab et al[4] first found activated microglia (increased spinal immune reactivity for OX-42) in a rat model with chronic visceral hyperalgesia, induced by neonatal colon irritation by repeated colorectal distension using an angioplasty balloon; the model could exhibit both peripheral and central sensitization. Using minocycline to interrupt microglia activation and its associated release of proinflammatory mediators would reverse the visceral pain behavior and restore normal processing of neuronal information along the visceral pain pathway in adulthood.

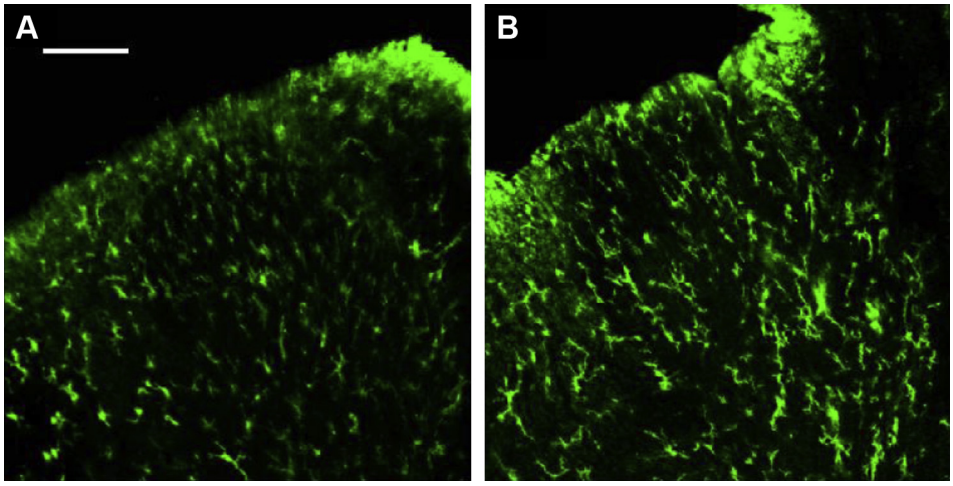

Fig. 1. Microglia activity (OX-42 immunofluorescence statin) in the spinal dorsal horn of rats in (A) resting status; and (B) trinitrobenzene sulfonic acid (TNBS) intraductal injection-induced chronic pancreatitis (scale bar = 100 μm).

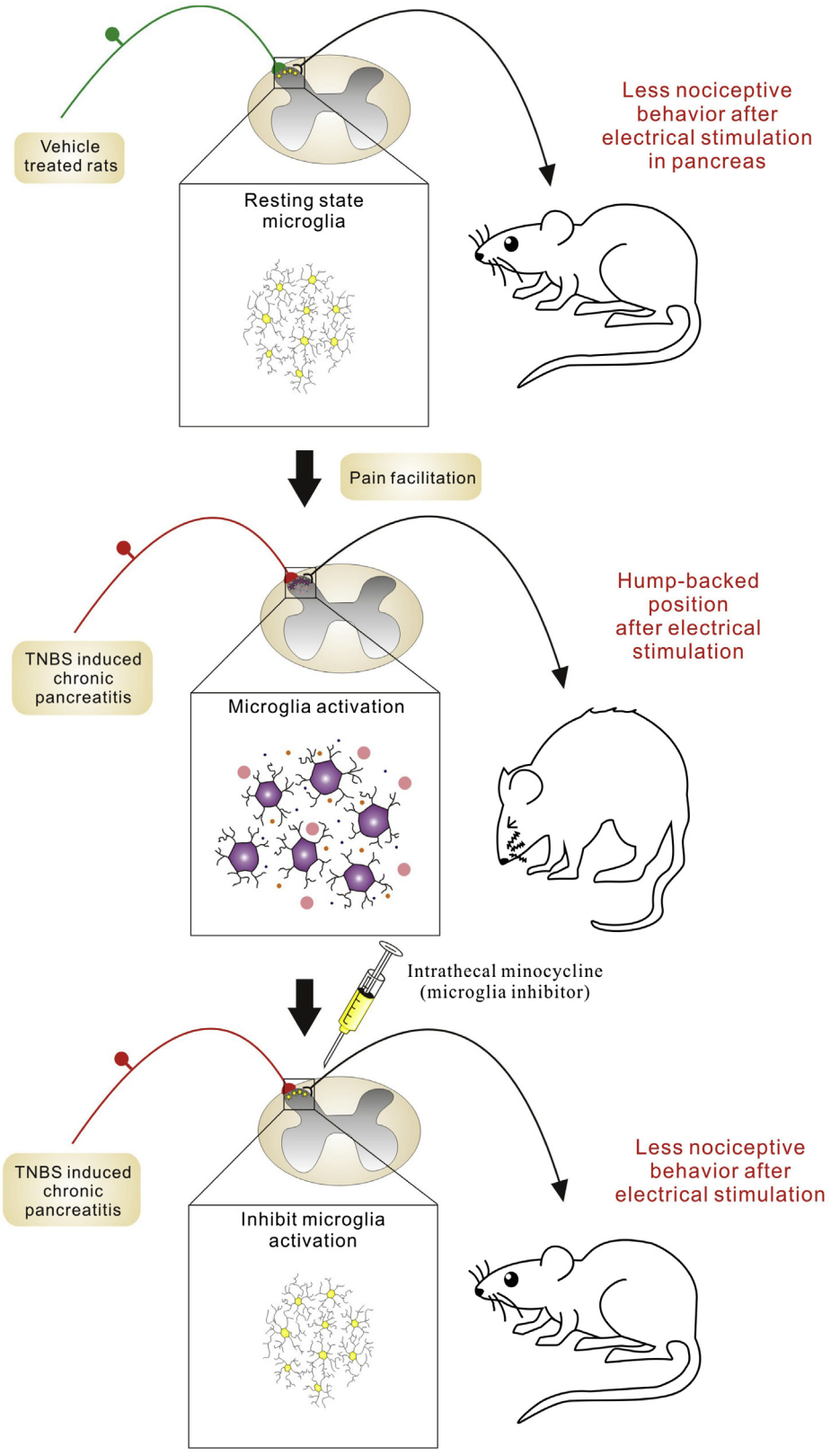

Fig. 2. Schematic illustration of the role of spinal microglia in mediating visceral hyperalgesia in a rat model of trinitrobenzene sulfonic acid (TNBS)-intraductal injection to induce chronic pancreatitis (CP). Minocycline can inhibit and prevent the microglia activation and the associated visceral hyperalgesia in TNBS-induced CP rat model.

In a model with trinitrobenzene sulfonic acid (TNBS) intracolonic instillation to induce severe gut inflammation, marked microglia activation and increased levels of tumor necrosis factor-α were noted in the hippocampus.[51] Although nociceptive behavior was not recorded in this report, visceral hyperalgesia is expected to be observed in the TNBS-treated rats.[52] This study also provided evidence of supraspinal activation of microglia upon gut inflammation, although its role in mediating visceral hyperalgesia remains to be clarified.

In another "non-inflammatory" model of visceral hyperalgesia, Bradesi et al[44] found that chronic psychological stress (water avoidance 1 hour/day for 10 consecutive days) can also lead to microglia activation in the lumbar spinal cord. Increased spinal immunoreactivity of P-p38 MAPK in OX42 positive cells was also noted from stressed rats, which can be blocked by minocycline. They also demonstrated that the stress-induced visceral hyperalgesia can be reversed by minocycline or the p38 inhibitor, SB203580. Furthermore, spinal injection of FKN (a microglia activator) in naïve rats can also induce visceral hyperalgesia, and this finding further supports the role of spinal microglia in mediating this stress-induced visceral hyperalgesia.

Recently, we first showed that activation of spinal microglia can play a key role in both initiating and maintaining visceral hyperalgesia in a TNBS-induced CP rat model.[53] The spinal microglia showed morphological changes (ameboid cells) with an increase in OX42 staining in the dorsal horn of the thoracic spinal cord in CP rats (Fig. 1). P-p38 levels were also increased in CP rats and colocalized with OX42-positive cells, but in neither NeuN-positive (neuron) nor GFAP-positive (astrocyte) cells. Intrathecal injection of minocycline would not only reverse, but also prevent the increase of nocifensive behaviors and P-p38 levels in CP rats (Fig. 2). FKN would induce visceral hyperalgesia in non-CP rats, which was also blocked by minocycline. We also found that the spinal Iκ-B level was significantly decreased after TNBS-induced pancreatitis; the Iκ-B level reversed to normal after minocycline treatment (unpublished data). This finding suggests the importance of NF-κB signaling pathway in microglia-mediated visceral nociception. We further noted that the spinal level of P2X7R, colocalized with OX-42, was significantly increased after CP (unpublished data). Pharmacological or genetic manipulation of P2X7 receptor by administration of a P2X7 antagonist (Brilliant Blue G) and siRNA to P2X7R would significantly reduce the visceral hyperalgesia in CP rats. These results indicate an important role of P2X7R on spinal microglia in mediating visceral hyperalgesia in a CP rat model. Taken together the current data, although limited when compared with available data from somatic pain studies, strongly supports the role of spinal microglia in the transmission of visceral nociception. Although some differences do exist in the pain process between visceral and somatic pain, spinal microglia may play an important and common role in the pathogenesis of both types of pain.

In conclusion, chronic visceral pain from FGID or CP creates a significant burden on society, as well as on individual patients. Because of unclear pathogenesis and poorly identified targets, the current treatment for chronic visceral pain has

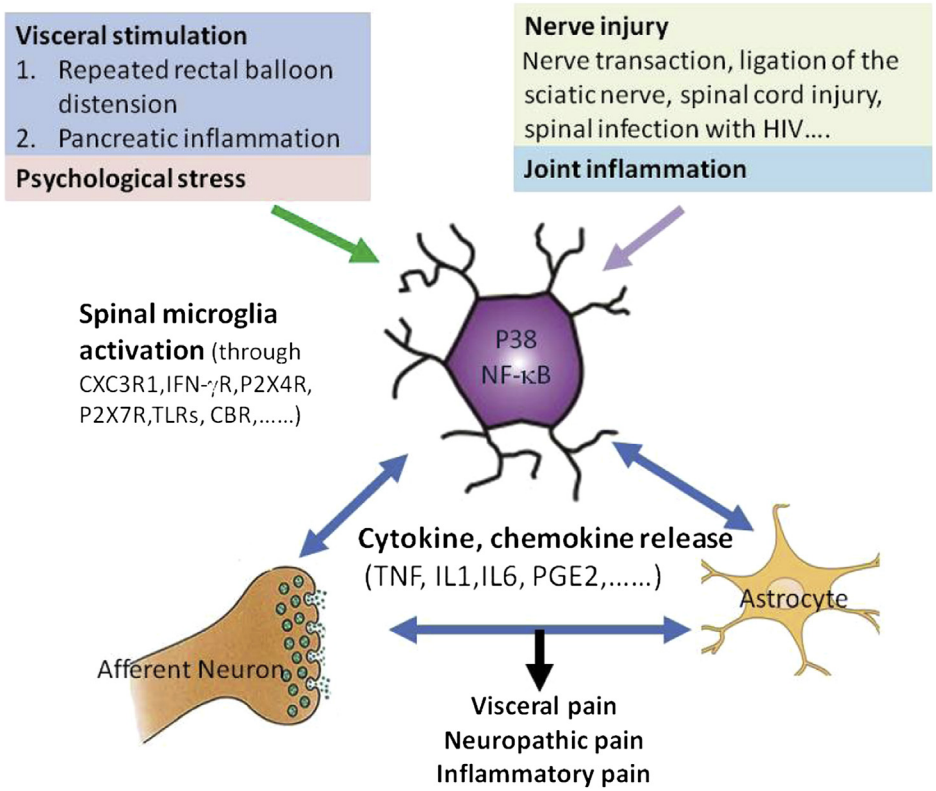

Fig. 3. Schematic illustration of the molecular and cellular mechanisms in the activation of spinal microglia in visceral, neuropathic, and inflammatory pain.

remained unsatisfactory. Recent preclinical studies suggested the involvement of microglia in the development and maintenance of chronic visceral and somatic pain (Fig. 3). Further studies focusing on microglia (such as tetracycline or Brilliant Blue G) during nociception may provide a promising strategy to identify novel targets for the treatment of chronic visceral pain in those patients with FGID or CP.

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