Enhancement of opioid-induced acute analgesia by targeting the peripheral MMP-9-dependent neuro-glial signaling

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Background

Opioids are the standard treatments for patients suffering from moderate to severe acute pain. They are mainly used during interventional procedures and as postoperative acute analgesia. Especially, the number of noninvasive and minimally invasive procedures that require a single systemic injection of opioids, such fentanyl and morphine, has grown exponentially over the last several decades. Practitioners performing interventional procedures include: gastroenterologists for endoscopies and colonoscopies; radiologists for imaging and interventional procedures; emergency department physicians for various procedures performed in the emergency room suite; internists, pediatricians, and family practitioners for biopsies and other office-based procedures; and anesthesiologists for high-risk operative and diagnostic procedures. Despite this increase in the number of physicians using opioids and their high efficacy, actions of opioids are not yet fully understood.

It is thought that opioid agonists, such as morphine, carry out their analgesic effects by actions at both the spinal and supraspinal levels (such as in the periaqueductal gray). However, opioid receptors are expressed throughout the central nervous system (CNS) and peripheral nervous system (PNS), and it stands to reason that opioid agonists may well be acting through both. The analgesic ability of systemically administered opioids is largely carried out through their actions on the PNS [1] and the activation of opioid receptors on sensory neurons decreases spontaneous neuronal activity. Intriguingly, several lines of evidence suggest that, in addition to antinociceptive actions, opioids also produce paradoxical excitatory and hyperalgesic effects involving not only neuronal pathways [2], but also immune responses in the CNS and PNS. In particular, chronic opioid exposure induces profound changes in spinal cord glial cells such as microglia and astrocytes [3] and, upon activation, spinal glial cells are capable of expressing a multitude of proinflammatory proteins including interleukin-1β (IL-1β). Interestingly, IL-1β has been shown to counteract opioid-induced analgesia after both chronic and acute administration of morphine, and administration of an interleukin-1 receptor (IL-1R) antagonist potentiates opioid-induced acute analgesia [4, 5]. Furthermore, genetic polymorphism of IL-1R has been implicated in the variation in postoperative morphine consumption [6].

To date, most studies exploring the proinflammatory responses to opioids have focused on chronic effects in CNS immune signalling. However, we have recently demonstrated for the first time that systemic acute morphine exposure activates an immune response in dorsal root ganglia (DRG), which is driven by a synergy between sensory neurons and satellite glial cells [7, 8]. Satellite glial cells (SGCs) are peripheral glial cells that tightly enwrap the cell bodies of sensory neurons in DRG and promptly react to chemical and electrical changes in the neuronal soma to regulate their homeostasis and excitability. Importantly, SGCs are involved in every clinically relevant animal model of chronic pain. Upon activation they display an increase of the glial fibrillary acidic protein (GFAP) and release an array of neuroexcitatory substances such as the proinflammatory cytokine IL-1β [9].

Matrix metalloproteinases (MMPs) are also important in chronic pain conditions [10]. MMPs consist of a large family of endopetidases that require Zn^{2+} for their enzyme activity and play a crucial role in inflammation through the cleavage of extracellular matrix proteins, chemokines and cytokines. MMP inhibitors have been developed to target different kinds of inflammation-related diseases including atherosclerosis, periodontitis, arthritis and cancer [11]. Recent studies illustrate that MMP-9 is a major player in chronic pain conditions through its role in processing IL -1β, as well as regulating the phenotype and proliferation of peripheral and central glial cells [12, 13].

Aims and Hypothesis

It is increasingly apparent that many patients do not achieve adequate pain control from existing opioid drugs, and the emergence of new multimodal opioid analgesia that addresses the activation of immune mechanisms in the PNS would provide patients with effective pain management and higher quality of life.

There is a growing recognition of the striking similarities in the immune signalling underlying chronic pain and opioid exposure [3]. Since MMP-9 is highly inducible and is released in the extracellular matrix in DRG after peripheral nerve injury (a model of chronic pain), where it modulates both neuronal and glial function, it became natural to question whether MMP-9 regulates the peripheral neuro-immune actions of opioids as well.

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Main Observations

Accumulating evidence suggests that novel neuro-immune signalling pathways play an important role in the development of opioid analgesia. We identified a novel role for MMP-9 in the activation of SGCs and subsequent release of the proinflammatory cytokine IL-1β in the DRG, which partially counteracts acute morphine-induced analgesia [7, 8].

To study the potential role of MMP-9 as a signaling molecule between sensory neurons and SGCs in DRG during acute analgesia, a single injection of morphine (10 mg/kg) was administered subcutaneously. First, we examined the role of MMP-9 in the regulation of acute morphine-induced analgesia. A single subcutaneous injection of morphine led to a rapid but transient (between 1 and 3 hours after the injection) increase in MMP-9 expression (fig. 1A) and activation. Importantly, there was no change of MMP-9 in the spinal cord. MMP-9 expression and activation returned to baseline levels 3 hours following morphine injection which was mediated by the tissue inhibitor of metalloproteinase 1 (TIMP-1), an endogenous MMP-9 inhibitor. MMP-9 is expressed in small- and medium-sized neurons that co-express the mu opioid receptor.

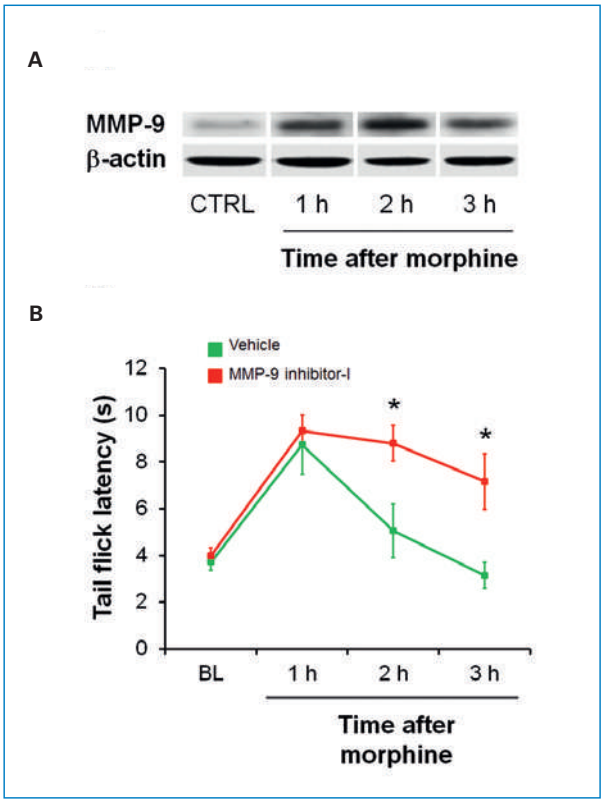

Figure 1
Characterization of MMP-9 as a mediator opposing acute analgesia after subcutaneous morphine injection (10 mg/kg). **(A)** Western blot showing the time course of MMP-9 expression levels in DRG. β-actin is the housekeeping protein used for normalization. CTRL = control. **(B)** Behavior analysis showing that intrathecal injection of MMP-9 inhibitor-I (0.2 µg) can enhance acute morphine analgesia. BL = baseline; *P <0.05, compared with vehicle group, Student's t-test; n = 8–10. Figure modified from reference [8].

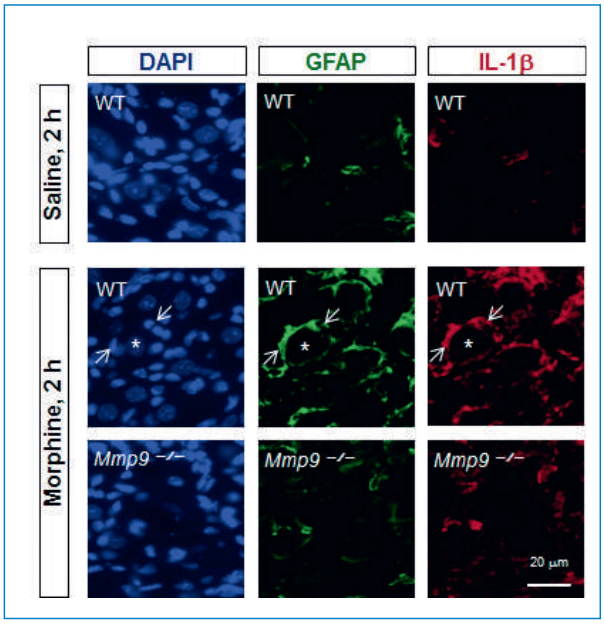

Figure 2
MMP-9 is required for subcutaneous morphine-induced GFAP and IL-1β expression in satellite glial cells of DRG. Left panels: DAPI nuclear staining of cells in DRG sections. Middle panels and right panels: Immunohistochemistry showing GFAP- and IL-1β-labeled SGCs in DAPI-stained DRG sections, respectively. Note that the morphine-induced GFAP and IL-1β increase at 2 h after morphine injection in wild-type mice (WT) is abrogated in MMP-9 knockout mice *(Mmp9$^{-/-}$)*. Star and arrows indicate a neurone (no staining) surrounded by SGCs, respectively. Figure modified from reference [7].

In dissociated neuronal DRG culture, MMP-9 expression was also increased by specific mu opioid receptor (MOR) agonist remifentanil and DAMGO ([D-Ala2, N-Me-Phe4, Gly5–ol]-enkephalin). In contrast, the opioid receptor antagonist naloxone and the MOR-selective antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2) blocked the morphine-induced MMP-9 up-regulation. To examine the effect of MMP-9 on acute morphine-induced analgesia, we employed several approaches to silencing MMP-9 expression, including MMP-9 knockout mice *(Mmp9$^{-/-}$)*, small interfering RNA against MMP-9 (siRNA, 3 µg), as well as administration of endogenous (TIMP-1, 0.2 µg) and synthetic (MMP-9 inhibitor-I, 0.2 µg) inhibitors of MMP-9. Acute opioid-induced analgesia was evaluated by measuring tail-flick latencies in response to the immersion of the mouse tail in hot water (52 °C). Consistently, the results from these different approaches all showed that MMP-9 inhibition and/or deletion can potentiate acute morphine-induced analgesia 2 hours after injection. Remarkably, synthetic MMP-9 inhibitor-I not only potentiated the morphine analgesia at 2 hours but also prolonged the analgesia for more than 3 hours (fig. 1B).

To further investigate the molecular and cellular mechanisms by which MMP-9 affects acute opioid analgesia, we studied the immune signaling in DRG. MMP-9 has been shown to induce glial cell activation and the release of proinflammatory molecules in the PNS [12,13]. Similarly, we found that acute subcutaneous morphine injection induced robust SGCs responses, as shown by an increase in GFAP transcriptional and protein expressions in the DRG but not the spinal cord. This mor-

phine-induced GFAP expression was transient and paralleled the expression of MMP-9, peaking at 2 hours and declining after 3 hours. Similarly, IL-1β expression in SGCs and activity in the DRG were increased after acute morphine administration. Immunohistochemistry analyses revealed that strong neuronal MMP-9 expression correlated with intense immunostaining of GFAP in SGCs, as well as the morphine-induced increase of GFAP and IL-1β which was abolished in *Mmp9*$^{-/-}$ mice (fig. 2). Notably, the inhibition of IL-1β in DRG, but not the spinal cord, by siRNA significantly potentiates acute morphine-induced analgesia.

Together our results suggest that targeting the peripheral neuronal-glial signalling mechanisms could enhance opioid analgesia.

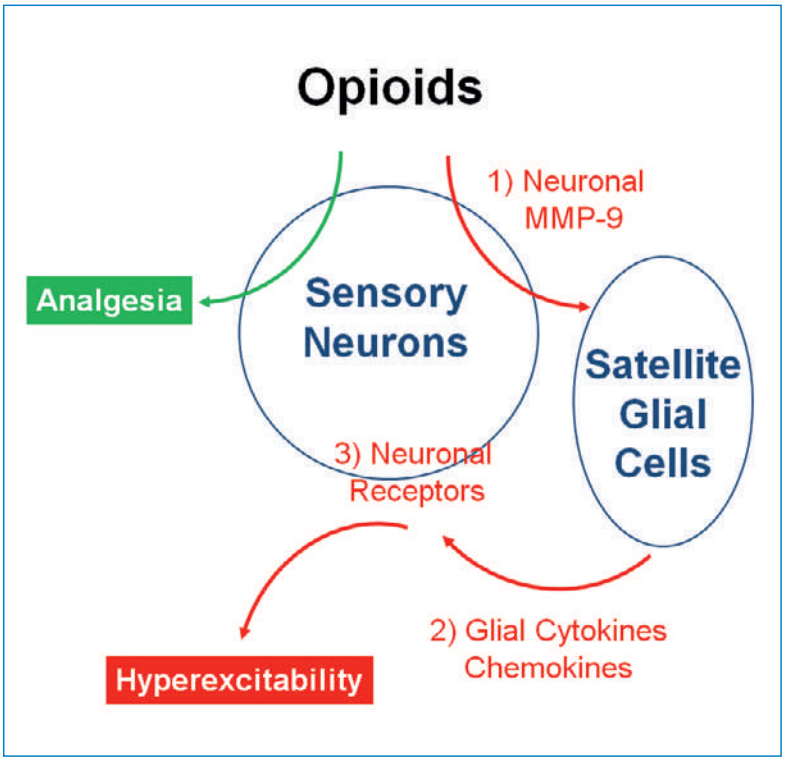

Figure 3
Schematic of proposed mechanism illustrating how morphine induces satellite glial cell activation and proinflammatory signalling in DRG to counteract morphine acute analgesia. Although morphine produces analgesia via the mu opioid receptor (green arrow), it also activates a detrimental signalling (red arrows) through the up-regulation of MMP-9 in sensory neurones (Step-1). Extracellular release of MMP-9 induces proinflammatory responses (i.e., production of cytokines and chemokines) from SGCs (Step-2). Finally, SGC-proinflammatory molecules can stimulate neuronal receptors to increase the excitability of the sensory neurones counteracting and shortening acute morphine analgesia (Step-3).

Table 1
Clinically relevant drugs used in animal models to improve opioid-induced acute analgesia.

Targeted mechanism	Drug
Glial cell activation	Minocycline [4], ibudilast [16], MMP-9 inhibitor I [8], TIMP-1 [8], siRNA targeting MMP-9 [8], LPS-RS [17], (+)-naloxone [17], (–)-naloxone [17], ultra-low (–)-opioid antagonists [18], TIRAP inhibitor [17], p-38 MAPK inhibitor [4].
Proinflammatory cytokine signalling	IL-1 receptor antagonist [4], siRNA targeting IL-1β [7], TNF soluble receptor [4], IL-6 neutralising antibody [4], IL-10 [5].

Conclusions and Perspectives

We provided several lines of evidence for not only the key role of MMP-9 in acute opioid analgesia, but also its machanism through the activation of satellite glial cells and the proinflammatory molecule IL-1β in the DRG (fig. 3). Targeting the upstream MMP-9 expression and immune activation in DRGs would provide considerable advantages such as higher drug accessibility outside the blood–brain barrier, the limitation of CNS-related side effects, and the prevention of glial cell activation and widespread of inflammation. For instance, minocycline, a prescribed antibiotic, can potently inhibit glial activation and proliferation, but it is worthwhile adding that it is also an inhibitor of MMP-9 [14]. Interestingly, it has been shown that minocycline enhances acute analgesia induced by morphine [15]. Given the strength of several drugs in animal studies (table 1), it is time to capitalize on the advancements in our understanding of opioid-induced immune signalling in the PNS and CNS by conducting clinical research and trials to determine the impact of this mechanism in patients. Further investigations may identify new peripheral neuronal glia signaling targets used as a framework for achieving our aim of a more successful opioid-mediated management of clinical acute pain.

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Recommended Literature
– Berta T, Liu T, Liu YC, Xu ZZ, Ji RR. Acute morphine activates satellite glial cells and up-regulates IL-1β in dorsal root ganglia in mice via matrix metalloprotease-9. Mol Pain. 2012;8:18.
– Liu YC, Berta T, Liu T, Tan PH, Ji RR. Acute morphine induces matrix metalloproteinase-9 up-regulation in primary sensory neurons to mask opioid-induced analgesia in mice. Mol Pain. 2012;8:19.

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