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REVIEW ARTICLE

Relationship Between Opioids and TLR4: Effects on Neuroglia

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ABSTRACT: 125 million opioid prescriptions were dispensed in America in 2023. With more people relying on opioids for their pain-relieving effects, evident from the increase in prescriptions from 2009, with 79.5 million prescriptions being dispensed then, unaware that they are building up tolerance and opioid-induced hyperalgesia (OIH) caused by taking the same drug. Opioids have the ability to bind to the toll-like receptor 4 (TLR4) in a manner similar to the lipopolysaccharide (LPS), irrespective of their stereochemistry. In this review, we focus on previous research aimed at answering the question: What effect does opioid binding to TLR4 have on the neuroglia? Long-term use of opioids can result in OIH. This mostly occurs because of glial cell activation and the opioids binding to the TLR4, reducing the analgesic efficacy of the drug. This suggests that focusing on blocking or reducing glial cell activation, or perhaps blocking opioids from binding to the TLR4 by using antagonists, might reduce pain sensitivity and increase the pain-relieving effects.

KEYWORDS: Cellular and Molecular Biology, Genetics, Glia, TLR4, Opioid Induced Hyperalgesia, Neuroinflammation.

Introduction

The pharmacology and treatment of pain have a long and complicated history. The first documented treatment was the use of opium poppy extracts around 1550 BCE.[1,2] Over time, opium and its compounds became the foundation of pain-relieving treatments. However, while it was recognized for its potential, its addictive properties and risk of misuse became more evident. The risk of opioid overdose has led to an increased exploration for more effective treatments to relieve pain, increase significantly. However, pain remains a public health challenge as nearly two-thirds of pain patients experience little to no relief from the available treatments.[3] Although opioids are widely used clinically to relieve pain, they are also able to induce inflammation in the brain and modulate the immune system in the body. Opioids induce the release of high mobility group box 1 protein (HMGB1), a danger-associated molecular pattern (DAMP) that also has the ability to activate the TLR4, triggering a signaling cascade of pro-inflammatory cytokines and chemokines.[4] In response to an infection, opioids can impair the number of macrophages responding to it.[5] Opioids can also modulate the release of hypothalamic-pituitary-adrenal (HPA) axis hormones, which in turn are able to regulate glucocorticoids, known for playing an important role in regulating cellular immune response.[6] Can blocking the opioids from binding to the TLR4 or blocking the upregulation of the DAMPs reduce immune suppression? Will it reduce the number of inflammatory cytokines released? The mechanisms that play a role in the immunosuppressive effects of opioids are still unknown.[4] Further research could help bridge the gap between chronic use of opioids and immune dysfunction.[7]

Therefore, the purpose of this review is to examine the relationship between opioids and toll-like receptor 4 (TLR4) and its potential impact on the neuroglia. TLR4 is a part of the Toll-like receptor family (TLR), a pattern recognition receptor specific to both intracellular and extracellular regions.[4] The TLR4 can activate the innate immune response through the detection of pathogen-associated molecular patterns (PAMPs), such as those from bacteria or viruses, and danger-associated molecular patterns (DAMPs) emerging from endogenous signals indicating cell death.[4] TLR4 is usually activated by its natural ligand, lipopolysaccharide (LPS), which requires the involvement of several additional proteins, including lipopolysaccharide-binding protein (LBP), cluster of differentiation 14 (CD14), and myeloid differentiation protein 2 (MD-2).[7]

This subsequently activates the My-D88 signaling pathway, which induces transcription factors such as NF-κB, IRF5, AP-1, and CREB, enabling the formation of pro-inflammatory cytokines. Opioids such as morphine were found to release glia-derived proinflammatory cytokines by binding to the MD-2 accessory protein, which plays a key role in neuroinflammation.[8] It has been widely documented to induce nociceptive sensitization known as opioid induced hyperalgesia (OIH); a condition where long-term opioid use leads to an increased sensitivity to pain, even the pain that was not originally present or was previously controlled by opioids.[9] Observed primarily in patients with chronic pain, where it has been enhanced by an underlying pain condition.[9]

Discussion

Mechanisms underlying morphine tolerance and allodynia:. Opioids, like morphine, can activate the spinal glial cells — The main types of glial cells in the spinal cord are astrocytes, oligodendrocytes, and microglia —, which then induces a strong proinflammatory response (causing inflammation) that rejects both acute and chronic pain relief provided by the opioid.[8] The proinflammatory response, as a result of TLR4 signaling in the ventrolateral periaqueductal gray (vIPAG) — a specific region within the brain's periaqueductal gray (PAG) that plays a crucial role in pain modulation and emotional responses — can also lead to the development of opioid induced hyperalgesia

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(OIH) and allodynia, a condition where non-painful stimuli are perceived as painful; The pathway by which this happens is still unknown.

Several pathways have been explored to better understand the interaction between opioids and spinal glial cells, including mu opioid receptor (MOR) decoupling, internalization and downregulation, enhanced N-methyl-D-aspartate receptor (NMDAR) activity, reduced glutamate transporter expression, and increased nitric oxide production.[3] Although these pathways are mainly for neuronal adaptations, additional research has focused on understanding the relationship between these processes and the effect they have on opioid tolerance. Evidence suggests that cytokines released by glial cells increase with the amount of morphine administered,[10] proposing the idea that these excitatory mediators are critical in the development of morphine tolerance. Thus, suggesting that inhibiting these cytokines could reduce the development of tolerance. However, it is also known that these inflammatory cytokines are involved in normal immune function and brain homeostasis; inhibiting these cytokines could disrupt these processes. This shows that simply targeting the cytokines wouldn't be the best solution to reverse the effects of morphine tolerance. Given the relationship between opioids and glial cells, it is understood that the production of proinflammatory cytokines opposes the chronic and acute analgesic effects of opioids.[3]

Researchers have found that morphine can bind to the neuronal MORs, which are primarily found on the GABAergic neurons, resulting in analgesia and tolerance. The pathway by which this happens is shown in Figure 1. In the vlPAG, activation of the MORs reduces the inhibition of GABAergic neurons to the PAG-RVM pathway; the neural pathway connecting the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM), resulting in a net excitation of the neurons in the spinal cord. Parallel glial activation intensifies the excitability of the MOR neurons, consequently reducing the analgesic effect of morphine and building up tolerance.[11]

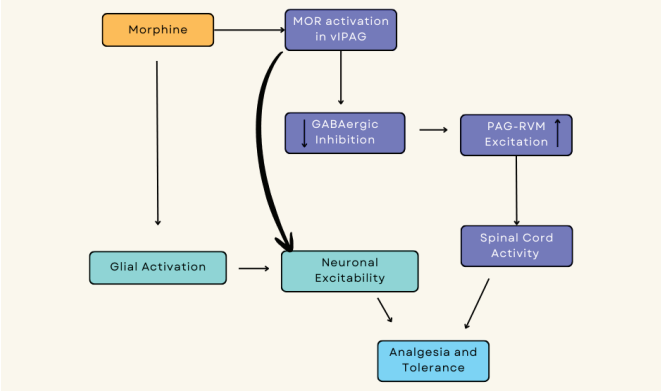

Figure 1: A pathway diagram demonstrating how the binding of morphine induces analgesia and tolerance. The light blue boxes highlight the neuronal pathway, the orange boxes highlight the glial pathway, and the red box highlights the outcome. Morphine first binds to the mu opioid receptor (MOR) in vlPAG, which leads to the inhibition of the GABAergic neurons, leading to the increased net excitation of PAG-RVM. The activation of the MOR also affects the neuronal excitability, just like the glial activation. The neuronal excitability and the excitation of the spinal cord enhance tolerance and decrease analgesia. Understanding that the pathway through which these effects take place involves the vlPAG, GABAergic neurons, and the PAG-RVM.

TLR4 signaling in opioid-induced neuroinflammation and its role in addiction:

A study showed that opioids such as morphine interact with the MD2 protein.[14] Once TLR4 is activated by opioids, it increases the amount of proinflammatory cytokines and chemokines released, altering the neuronal plasticity.[15] Opioid-induced glial activation is considered to contribute to the reinforcement of reward-like effects, achieved by binding to TLR4. The known neuronal mechanism in relation to opioid addiction involves the suppression of the GABAergic tone on the dopaminergic reward pathway from the ventral tegmental area (VTA) to the nucleus accumbens (NA), leading to enhanced dopamine release, hence the reward-like feeling.[15] Tumor necrosis factor-alpha (TNFα), a downstream effector of TLR4 signaling, demonstrated that it can mediate morphine-induced neuroinflammation, and inhibiting it reduced TLR4-dependent inflammatory responses.[12] Like TNFα, activating TLR4 also increases the amount of interleukin-1β (IL-1β) expression.[16] The role of IL-1β is to mediate long-term potentiation (LTP), which plays a role in memory.[17] IL-1β can inhibit the glial glutamate transporter, which reduces the amount of glutamate, disrupting the glutamate-glutamine cycle-dependent GABA synthesis. The processes stated above play a key role in synaptic plasticity, which may affect TLR4-related drug actions.[15] TLR4-mediated central immune response to opioids such as morphine can only function with respect to well-constituted reward neuronal pathways, since immune signaling alone cannot induce behavioral changes.[18] The neuroinflammation caused by opioids has been associated with morphine analgesia, dependence, tolerance, and withdrawal effects.[12] Enhanced morphine induced analgesia was observed in TLR4/MD2 knockout mice, which shows that interference with TLR4/MD2 signaling inhibits morphine induced proinflammatory responses. However, some studies oppose the idea that TLR4 has much to do with opioid addiction.[13] This is backed by the findings of Stevens and colleagues that showed morphine inhibits LPS-induced activation of TLR4 in a concentration-dependent manner. Keeping in mind how two different pathways are being considered for the same effect, this begs the question of what other signaling pathways are involved in this interaction, opening areas to further research.

From this, we can understand that opioid induced neuroinflammation is mediated partially through the TLR4 signaling, which in turn contributes to the analgesic, addictive, and inflammatory effects of the opioid by allowing the release of pro-inflammatory cytokines, as stated above. However, it is also good to note that contrasting evidence of TLR4's involvement in opioid addiction suggests that there are other pathways involved.

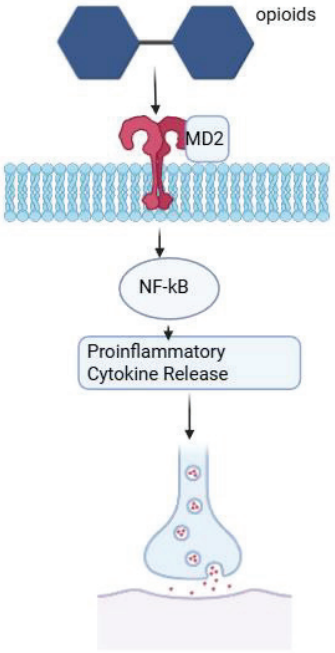

Figure 2: Shows the opioid binding to the accessory protein MD2, which results in the downstream signaling of the NF-kB. This leads to the release of proinflammatory cytokines.

Role of TLR4 activation and M3G in mediating opioid induced hyperalgesia and tolerance:

Depending on the location of the activation, TLR4 activated by opioids can cause neuroinflammation,[19] leading to opioid induced hyperalgesia, dependence, reward, and reinforcement.[20] The effects of pharmacological blocking of TLR4 were evaluated by Gabr *et al.* The evaluation brought to light that the analgesic efficacy of morphine improved when morphine was administered along with a TLR4 blocker or a peptide that blocks the Toll-Interleukin-1 receptor domain.

An earlier paper suggests that morphine-3-glucuronide (M3G) can elicit pain enhancement and develop hyperalgesia.[22] However, the details of the mechanism are still unknown.[23] Furthermore, an *in vitro* study showed that M3G can activate the TLR4, suggesting that the pain enhancement of M3G could be a result of the proinflammatory response. Enhanced allodynia and thermal hyperalgesia were observed when M3G (0.75 μg) was administered intrathecally — a route of drug administration where a substance is injected into the cerebrospinal fluid (CSF) (the fluid surrounding the brain and spinal cord) — *in vivo*. This effect was also reduced when M3G was co-administered with glial inhibitors, proinflammatory cytokine inhibitors, and either isomer of naloxone.[24] Figure 2 shows the experimental setup used to show how these conclusions were drawn. Studies have shown that repeated use of morphine leads to increased activation of the glial cells in the vlPAG.[25] Further research showed that TLR4 signaling in these cells played a role in developing opioid tolerance. The administration of glial inhibitors, proinflammatory cytokine inhibitors, or naloxone into the vlPAG blocked the development of opioid tolerance. However, the administration of morphine in vlPAG induced tolerance.[10]

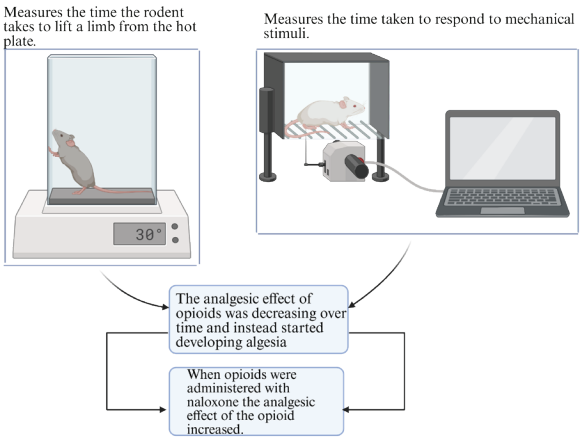

Figure 3: The experimental setup used to measure the time taken for a response to be recorded for both the thermal and mechanical stimuli. 2 groups of mice were tested, the first group being rodents administered with only the opioid, in this case M3G, and the second group being rodents administered with both the opioid, M3G, and naloxone, an opioid antagonist. The first group of rodents showed decreasing analgesic efficacy of the drug and the development of analgesia over time as more M3G was administered. However, the second group of rodents showed increasing analgesic efficacy of M3G compared to the first group. Suggesting that the administration of opioids with their antagonist could increase their analgesic efficacy. However, this investigation only focused on the effects of opioids on sensitivity and how the administration of an antagonist helped with the efficacy; it's important to note that this method did not highlight the effects… had on the immune system or brain homeostasis. Activation of TLR4 by opioids induces neuroinflammation, tolerance, hyperalgesia, and dependence. It can be seen that blocking the TLR4 increases morphine's analgesic effects. We can also understand through the study above that the proinflammatory pathways activated by the binding of opioids to the TLR4 can be diminished by cytokine or glial inhibitors.

Downstream NF-kB and MAPK activation in neuroinflammation and tolerance:

Opioid receptor agonists are able to activate the TLR4 without the use of additional proteins, even without the need for LPS.[26] This ability, in particular, is also exhibited by endogenous opioids, a few of which originate from immune cells.[27] Traditionally, the interaction between opioids and their receptors is stereoselective. However, the binding of opioids to the TLR4 is non-stereoselective, meaning all stereoisomers of the opioid can bind to the TLR4. Uniquely, Morphine-3-glucuronic acid (M3G) — a morphine metabolite processed in the liver lacking affinity to bind to opioid receptors — can activate the TLR4. HGMB1, an endogenous antagonist of TLR4, interacts with danger signals produced both exogenously and endogenously to induce inflammation. HGMB1 is said to be activated by both immune cells and neuronal cells.[28] Surprisingly, the activation of the TLR4 has been linked to the morphine-induced HMGB1 production.[27,29] Increased levels of HMGB1 in our body have been thought to play a role in the development of inflammation, tolerance, hyperalgesia, and allodynia.[26]

MD2, a key component of the TLR4 signaling, detects the LPS and initiates the innate immune response. Opioids bind to the MD2, activating the nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB), a protein complex that plays a crucial role in regulating gene transcription, cytokine production, and cell survival. Inducing the release of

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proinflammatory cytokines such as TNFα and IL-1β [30] all the while activating the MAPK pathway — a crucial signaling cascade in cells that transmits extracellular signals to the nucleus — allowing for the development of inflammation.[26] This suggests a possible interaction between immune cells and glial cells.[19] The interaction between opioids and the TLR4 may contribute to the development of hyperalgesia, allodynia, and tolerance, most of which are also linked with opioid-induced activation of proinflammatory glia.[27,31] Studies suggest that opioid-induced immune response and the key role played by neuroglia may be the reason behind the drugs' reduced efficacy.[28] Activating the transmembrane TLR4 initiates the innate immune response, leading to nuclear factor kappa B (NF-κB) activation, a protein complex that plays a crucial role in regulating gene expression, particularly in response to inflammation, immunity, and cell survival; while simultaneously activating the transcription of proinflammatory cytokines in macrophages, monocytes, and glial cells.[27] This highlights the decreasing effect of opioids.

Previous studies have shown that continuous morphine administration enhances HMGB1 release and induces mechanical allodynia — a condition where a non-painful stimulus, like light touch or pressure, is perceived as painful — that can continue even after the morphine treatment has been stopped.[27] In regard to this, increased IL-1β from activated spinal microglia has been noted, in addition to the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome; a multi-protein complex that plays a crucial role in innate immunity by activating caspase-1 and the inflammatory cytokines interleukin (IL)-1β and IL-18.[32] Studies in TLR4 knockout mice have shown the involvement of TLR4 in reducing the pain relieving effect of the opioid. Indicating that the anti-analgesic effect of the opioids may not be entirely mediated through the TLR4, but it can support or enhance the effect.[15,27] Opioids and their metabolites can non-stereospecifically bind to the TLR4, activating the NF-κB and MAPK pathways.

Conclusion

The studies and the evidence stated above show how opioids bind to the TLR4 to initiate the proinflammatory signaling. This interaction leads to opioid-induced hyperalgesia, tolerance, and allodynia. The mechanisms that underlie these consequences are TNF-α and IL-1β, HMGB1-mediated amplification of inflammation, and NLRP3 inflammasome activation. While we now realize that TLR4 is not the sole mediator of these effects, it does play a role in enhancing the effects caused by opioids.

Targeting the TLR4 signaling directly or through inhibition of glial and cytokine pathways could be a potential solution to the above-stated consequences or increase the analgesic efficacy of the opioid. Future direction for this research could focus on distinguishing the contributions of TLR4 in the development of tolerance, OIH, and allodynia, and the contributions of the pathways and their link to the effects. Additionally, the direction could be steered towards the hormones of the immune system and using developing genetic technology to engineer them to combat the consequences of opioid misuse. A good idea would be to use the DREADDS technology to find specifically which neuronal pathways play a key role in leading to opioid misuse and target the proteins in those pathways to reduce the consequences of opioid misuse. This review is limited in the number of clinical trials referred to.

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