Review Article

The role of Toll-like receptors in pain control

Parisa Sezari[1*]

Abstract

Toll like receptors are expressed primarily by immune cells and are known to be part of the innate immune system. In the past decade involvement of TLRs in several physiologic and pathologic pathways has been proved. Pain transmission via glial activation is one of such interesting fields. Both pathological pain states and treatment have also been shown to be related to TLR-related pathways. Opioid agonists are found to possess TLR4 agonistic effects and glial activity. Targeting TLRs could be a novel method for treatment of neuropathic pain. Moreover attenuation of glial activation by the aim of selective TLR antagonistic drugs, may become a preferred way of separating the beneficial (analgesia) and unwanted effects of opioids, improving their safety and efficacy.

Keywords: Toll like receptors, microglia, analgesia, withdrawal, pain control

Please cite this article as: Sezari P. The role of Toll-like receptors in pain control. J Cell Mol Anesth. 2017;2(1):24-9.

1. Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author:
Parisa Sezari, Assistant Professor, Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tel/Fax: (+98) 21 2243 2572.
E-mail: parissasezari@yahoo.com

What are TLRs

Toll like receptors (TLRs) are a class of proteins that are among key elements of innate immunology structure; so, they are seen in dendritic cells, macrophages, neutrophls, mucosal epithelial cells, and other cells of innate immunology. General understanding of the immune system is based on that "self and foreign" must be distinguished so self paricles are tolerated and the aliens can be defeated. Triggering innate or adaptive immune responses and recognition of foreign antigens are processed through several pathways. Innate immunity involves binding of the pathogen by so-called pattern recognition receptors (PRR) to enablefurther danger signaling (1).Within the PRR, the toll like receptor family is specifically involved in immune cell activation. Toll like receptors (TLRs) are pattern recognition receptors, which means they recognize PAMPs (Pathogen-Associated Molecular Patterns) and DAMPs (Damage-Associated Molecular Patterns) from tolerable self antigens.Whenever a pathogenic molecule traspasses the physiologic barriers and enters body environment, it will be encountered wih TLRs, which then activate the innate immune system. (2-4) Interestingly, TLRs also recognize endogenous ligands on the injured cells or over the surface of tissue fragments, representing endogenous danger signals. Each type pf TLRs binds to several specific ligands, and so a more specialized pathway will be activated through each (5).

TLRs are recognized from TLR1 to TLR13; however, TLR1 to TLR11 are distinguished in human being (6). TLR1 is a known receptor for Bacterial products such as tri-acyl lipopeptides,while TLR 2 recognizes gram positive bacterial products and several viral proteins (7). TLR3 only recognizes Viral

double-stranded RNA,wheras TLR9 is able to detect both viral and bacterial DNA (8). TLR4 is mainly known for detecting endotoxin (lipopolysaccharyde or LPS), but it also recognizes host cell stress and damage products like cytoplasmin and/or nuclear particles like DNA,RNA and heat shock proteins. Extracellular matrix breakdown products such as hyaluronan, heparan sulfate, fibrinogen, or the fibronectin end products can also activate TLR4. (1, 9).

TLR4

LPS can cause endotoxic shock by inducing the release of proinflammatory cytokines and chemokines from immune and non-immune cells. In humans, TLR4 mutations are also associated with impaired responsiveness to LPS It was recently suggested that endogenous ligands have shown to activate NFκB mobilization and mitogen-activated protein kinases (MAPK) pathways through their direct binding to TLR4. TLR4 recognizes endogenous molecules that are exposed during cellular injury and extracellular matrix remodeling, which means that TLR4 signaling may also be involved in activation of immune responses during independent of pathogen invasion.

TLRs and Pain

Pain processing is a dynamic chain of events, controlled by regulatory modulators. Under normal conditions, a painful stimulus is perceived via activation of "pain"-receptive sensory nerve fibers., also known as "nociceptors". The resultant action potentials conduct the information of potential or actual tissue injury through pain transmission neurons in the spinal cord dorsal horn. These neurons send the information to multiple sites within the brain where various aspects of the pain experience (sensation, analysis of meaning, emotional reactions, etc) are analyzed and responded to. Pain messages can be suppressed by drugs like morphine, transferred unaltered, or amplified under several pathologic conditions. When pathologic pain develops as a result of peripheral nerve injury, it have been attributed to a variety of neuronal changes, including altered excitability of sensory neurons, alterations in which neurotransmitters are synthesized and released by various sensory neurons, alterations in pain transmission neuron excitability via multiple changes in receptor and ion channel functions, etc. Several mechanisms are known in pain transmission and sensitization/inhibition (4).

Acute pain is transient and mostly resolves within weeks, while persistent pain can last for up to 3 to 6 months. (10) Both acute and persistent pain arise from tissue damage and/ or inflammation, and so the nociceptive transmission in these cases is not necessarily dysfunctional. Several well- identified signals trigger pain conduction following any painfol stimulant. Substance P which binds to the neurokinin 1 receptor (also excitatory amino acid glutamate, binding to AmPA (α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid) and NMDA (*N*-methyl-d-aspartate) receptors, calcitonin gene-related peptide (CGRP), galanin, vasoactive intestinal polypeptide and somatostatin, are some of well-known chemical signals (11, 12). The activity of these pain-projection neurons is also influenced by local inhibitory interneurons in the spinal cord and by supraspinal mechanisms (4).

Although acute pain from an identifiable trauma is considered a symptom of tissue injury or disease, chronic and recurrent pain is a disease. Under normal conditions pain pathway is activated to induce protective responses which help to prevent further tissue damage. Treating clearly identifiable causes of pain normally leads to a resolution of such pain. When injury or inflammation is prolonged, ongoing excitation of nociceptive neurons results in chronic pain. Injured or inflamed tissue in the CNS can also sensitize neurons in the spinal cord, leading to chronic pain (4). These chemical and neural changes occur at the site of tissue injury in the body, either at the nerve endings of pain fibres or along their axons, and at first-order synapses, both pre- and postsynaptically in the dorsal horn of the spinal cord and/or in supra-spinal pain-processing areas. These well-characterized changes in neuronal and biochemical processing, from normal conditions to a pain-facilitatory state in the dorsal horn of the spinal cord, are collectively known as "central sensitization" (13). Several mechanisms that bring about central sensitization have been described. The best characterized involves a change in the function of

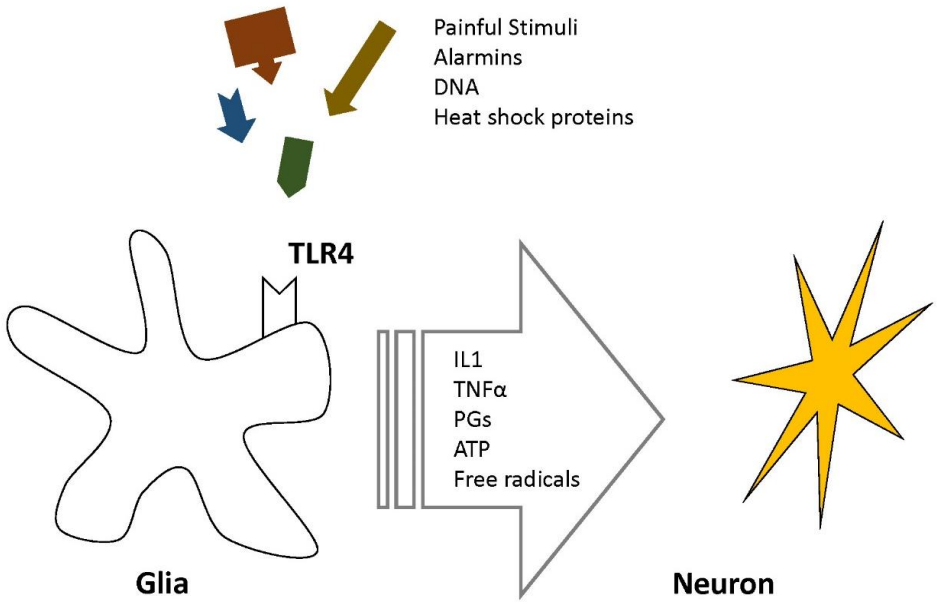

Figure 1. TLR4-mediated Neuronal Excitability. Several contributors like painful stimuli, exogenous materials (bacterial lipopolysaccharides), endogenous danger signals of cellular stress or damage (Alarmins), and also release of DNA and other self substances (normally hidden from immune system) will activate glia through their interaction with Toll like receptors. As a result glial cells secret several "neuroexcitatory pain enhancing substances",shown in the arrow.These mediators will initiate and augment neuronal excitation through several mechanisms (i.e. NMDA and AMPA receptor upregulation, GABA receptor downregulation, potassium outward current attenuation and sodium inward current enhancment).

neuronal NMDA (*N*-methyl-d-aspartate) receptors in the spinal cord dorsal horn (14). Chronic Pain is mediated by several neuronal and immune pahways. The interaction between neuronal and immune system in the CNS is known as "Central Immune Signaling".TLRs are the critical part of this signaling process, enhancig neuroexcitation via proinflammatory mediator release (2, 15).

TLR and glial activation

Altered activity of primary sensory nociceptors and/or spinal and/or brain neurons relevant to the pain pathway, leads to chronic neuropathic pain. Neuropathic pain is often associated with direct trauma or inflammation of peripheral nerves (16). Neuropathic pain initiates by sensory neuron injury, which activates spinal cord glia. Glial cells are important mediators in the pathological pain process. Although glia are well known for having a number of nurturing functions that are necessary for healthy neuronal communication, on strong activation they act as immunoresponsive cells or exert a neuroprotective effect. Glia can contribute to neuropathic-pain processing by releasing a number of glial and neuronal signalling molecules. Neuropathic pain is a fine example of glia roles in astrocytic activation (4).

Activation signaling is tought to be mostly started by TLR4 stimulatation, which then contributes in CNS immune sysem activation and proinflammatory cytokines release (2, 17, 18). TLR4 is expressed on the surface of glia but not on the neurons (19). It also has been demonstrated that mRNA for TLR4 in spinal cord is increased after L5 nerve transection (20).The activation of TLR2 and TLR4 induces the release of IL-1β, TNFα and IL-6, that leads to an excitatory positive feedback loop in pain pathway (21). Microglia are the first glial type to become activated and that their activation leads in turn to the recruitment of nearby astrocytes, so both cell types contribute to observed alterations in pain (22). astrocytes and microglia interact. Their released products can synergize, and substances released by one can activate the other. Regarding synergy,

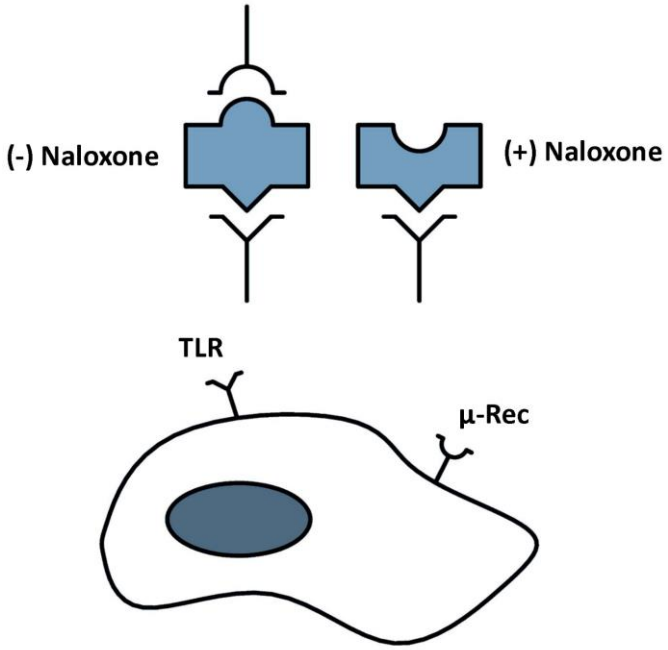

Figure 2. Non-Stereoselectivity of Toll like receptors for opioid agonists/antagonists. Classic opioid receptors expressed by neurons are highly stereoselective for binding (-)opioid isomers, while TLR4 (expressed predominantly on microglia) binds to both (+) and (-) isomers. This fact predicts that neuronally inactive (+)-opioid antagonists such as (+)-naloxone and (+)-naltrexone could be developed into clinically viable drugs for potentiating opioid analgesia and without altering analgesic effects.

proinflammatory cytokines can synergize with each other as well as with neurotransmitters and neuromodulators (23).Searching for the role of TLR4 in neuropathic pain development and treatment, it has also been observed that tricyclic compounds such as amitryptiline,commonly used in neuropathic pain management, has obvious TLR4 inhibitory activity while enhancing opiod analgesic effects (24).

TLRs and Opioids

Opioids have recently been shown to activate glia through a receptor that is distinct from the classical opioid receptor on neurons. This opioid-induced activation of glia induces them to release pro-inflammatory cytokines, which further suppresses opioid analgesia. Repeated exposure to opioids leads to enhanced pro-inflammatory cytokine release from glia. (25) Opioids not only suppress pain, they also activate endogenous counter-regulatory mechanisms that, for example, actively oppose opioid-induced pain suppression, enhance analgesic tolerance wherein repeated opioids lose their ability to suppress pain, and enhance dependence wherein organisms require continued opioid exposure to stave off drug withdrawal. (26) These modulatory controls have again been attributed to a variety if neuronal mechanisms, including release of endogenous anti-opioid peptides such as cholecystokinin, internalization and/or desensitization of opioid receptors, alterations in opioid receptor signaling cascades, and so on.Activation of TLR4 leads to downstream releae of inflammatory modulators including TNF-α and interleukin-1.Constantant low-level release of these modulators is thought to reduce the efficacy of opioid drug treatment with time, resulting in development of tolerance to opioid analgesic effects (27).

Side effects of extended opioid usage,and its known phnomena known as “hyperalgesia” and “allodynia”, may also be related to constatnt inflammatory mediators releae (28). Blockade of TNF-α and interleukin-1 actions is known to enhance the analgesic effects of opioid analgesics, and also to reduce the development of tolerance to them.The same results have been demonstrated wih drugs blocking TLR4.

Blocking TLR-dependent opioid-induced glial

activation enhances acute opioid analgesia and suppresses the development of opioid tolerance. Furthermore, suppressing glial activation suppresses the development of opioid dependence and withdrawal, suppresses opioid reward linked to drug abuse and suppresses other negative side effects, such as respiratory depression. Preventing glial activation in response to opioids represents a promising way to enhance the clinical efficacy of opioid drugs while decreasing their unwanted effects. It has been observed that inhibition of intratechal TLR4 by its antagonists enhances opoid anlgesia and suppresses neuropathic pain formation and decreses its severity (17). TLR4 is also importantly involved in glial dysregulation of opioid actions. Those studies demonstrate that, similarly to neuropathic pain, morphine upregulates TLR4 expression in microglia (29). Activation of glial TLR4 by opiod agonists triggers release of several cytokines and neurotransmitters, resembling inflammation state,and results in further opioid receptor tolerance. IL-1 and other proinflammatory cytokines oppose the ability of morphine to suppress pain, showing a round-way relationship between opioids and TLR pathway (30).

TLR4 has also been shown to be involved in the long-term side effects of opioid analgesic drugs.Various ligands of μ-opioid receptor has been tested and found to also possess agonistic and/or antagonistic effects on TLR4, with opioid agonists such as morphine being TLR4 agonists,while opioid antagonists such as naloxone were found to be antagonists of TLR4 (Figure 2). Taken together with the fact that neuropathic pain is created, at least in part, via activation of TLR4 contributing to microglial activation (17, 20), these data suggest that TLR4 may prove to be a target worth exploring for improving clinical pain control. Hutchinson et al. have shown these effects in several studies (17, 31, 32). Intrathecal administration of either the TLR4 receptor antagonist or intrathecal morphine produce analgesia (29). Considerably, several clinically relevant opioids including morphine, methadone, meperidine, and fentanyl are observed to activate TLR4 (29).

Clinical Implications

Recent discoveries in the role of the immune system in pathological pain states, including neuropathic pain could be a potential breakdown. Within the few past years, recognition of immune-like glial cells and their relation with the efficacy of opioids for pain control has been observed in animal models (32).

The potential implications of TLR4 in pain management are also vast. Many of the present possible treatments for pathologic pain are unsatisfactory. Most of pain treating drugs currently used target neurons. The discovery of glial regulatory role through TLR-dependent mechanisms is bringing up a whole new perspective in pain management strategies.

Glial TLR4 modulatory, impacts on the therapeutic effects of opioids has not yet been assessed in human. The fact that non-μ opioid receptors (TLRs) appear to mediate the glial activating effects of opioids suggests that the clinical efficacy of these analgesics can be improved in magnitude and duration by separating the glial activating effects of opioids from their pain suppressive ones. Also, dependence and withdrawal are unwanted consequence of opioid use which can occur in humans after even brief opioid use (33, 34). Coadministration of a “glial activation inhibitor” with an opioid might be a probable way to intensify analgesia, delay the development of tolerance, and decrease the incidence of opioid dependence or withdrawal.

Conclusion

As surgical procedures are growingin numbers worldwide, anesthesia techniques and drugs will evolve. Searching for more effective drugs with less side effects, TLR agonist/antagonists could have new role in modern anesthesia, both by their own specificities and their synergistic effects on other well-known analgesic drugs. Further studies can help us to learn more about their effects on other physiologic processes within body throughout anesthesia and surgery.

Acknowledgment

The author would like to acknowledge the staff of Anesthesiology Research Center, Shahid Beheshti

University of Medical Sciences, Tehran, Iran for their kind assistance.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

1. Anders HJ, Banas B, Schlondorff D. Signaling danger: toll-like receptors and their potential roles in kidney disease. J Am Soc Nephrol. 2004;15 (4):854-67.
2. Austin PJ, Moalem-Taylor G. The neuro-immune balance in neuropathic pain: involvement of inflammatory immune cells, immune-like glial cells and cytokines. J Neuroimmunol. 2010;229 (1-2):26-50.
3. Peirs C, Seal RP. Targeting Toll-like receptors to treat chronic pain. Nat Med. 2015;21 (11):1251-2.
4. Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. 2009;10 (1):23-36.
5. Ozinsky A, Smith KD, Hume D, Underhill DM. Co-operative induction of pro-inflammatory signaling by Toll-like receptors. J Endotoxin Res. 2000;6 (5):393-6.
6. Takeda K, Akira S. Regulation of innate immune responses by Toll-like receptors. Jpn J Infect Dis. 2001;54 (6):209-19.
7. Jin MS, Lee JO. Structures of the toll-like receptor family and its ligand complexes. Immunity. 2008;29 (2):182-91.
8. Kang JY, Lee JO. Structural biology of the Toll-like receptor family. Annu Rev Biochem. 2011;80:917-41.
9. Botos I, Segal DM, Davies DR. The structural biology of Toll-like receptors. Structure. 2011;19 (4):447-59.
10. Carr DB, Goudas LC. Acute pain. Lancet. 1999;353 (9169):2051-8.
11. Hunt SP, Mantyh PW. The molecular dynamics of pain control. Nat Rev Neurosci. 2001;2 (2):83-91.
12. Hucho T, Levine JD. Signaling pathways in sensitization: toward a nociceptor cell biology. Neuron. 2007;55 (3):365-76.
13. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000;288 (5472):1765-9.
14. Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. Anesth Analg. 2003;97 (4):1108-16.
15. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010;16 (11):1267-76.
16. Zimmermann M. Pathobiology of neuropathic pain. Eur J Pharmacol. 2001;429 (1-3):23-37.
17. Hutchinson MR, Zhang Y, Brown K, Coats BD, Shridhar M, Sholar PW, et al. Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). Eur J Neurosci. 2008;28 (1):20-9.
18. Buchanan MM, Hutchinson M, Watkins LR, Yin H. Toll-like receptor 4 in CNS pathologies. J Neurochem. 2010;114 (1):13-27.
19. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. Semin Immunol. 2007;19 (1):3-10.
20. Tanga FY, Raghavendra V, DeLeo JA. Quantitative real-time RT-PCR assessment of spinal microglial and astrocytic activation markers in a rat model of neuropathic pain. Neurochem Int. 2004;45 (2-3):397-407.
21. Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. Glia. 2002;40 (2):195-205.
22. Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain. 2005;115 (1-2):71-83.
23. Watkins LR, Hutchinson MR, Ledeboer A, Wieseler-Frank J, Milligan ED, Maier SF. Norman Cousins Lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids. Brain Behav Immun. 2007;21 (2):131-46.
24. Hutchinson MR, Loram LC, Zhang Y, Shridhar M, Rezvani N, Berkelhammer D, et al. Evidence that tricyclic small molecules may possess toll-like receptor and myeloid differentiation protein 2 activity. Neuroscience. 2010;168 (2):551-63.
25. Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, et al. A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. J Neurosci. 2004;24 (33):7353-65.
26. Hutchinson MR, Zhang Y, Shridhar M, Evans JH, Buchanan MM, Zhao TX, et al. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav Immun. 2010;24 (1):83-95.
27. Nicotra L, Loram LC, Watkins LR, Hutchinson MR. Toll-like receptors in chronic pain. Exp Neurol. 2012;234 (2):316-29.
28. Thomas J, Hutchinson MR. Exploring neuroinflammation as a potential avenue to improve the clinical efficacy of opioids. Expert Rev Neurother. 2012;12 (11):1311-24.
29. Hutchinson MR, Bland ST, Johnson KW, Rice KC, Maier SF, Watkins LR. Opioid-induced glial activation: mechanisms of activation and implications for opioid analgesia, dependence, and reward. ScientificWorldJournal. 2007;7:98-111.
30. Shavit Y, Wolf G, Goshen I, Livshits D, Yirmiya R. Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance. Pain. 2005;115 (1-2):50-9.
31. Watkins LR, Hutchinson MR, Rice KC, Maier SF. The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. Trends Pharmacol Sci. 2009;30 (11):581-91.
32. Watkins LR, Hutchinson MR, Milligan ED, Maier SF. "Listening" and "talking" to neurons: implications of immune activation for pain control and increasing the efficacy of opioids. Brain Res Rev. 2007;56 (1):148-69.
33. Savage SR. Opioid therapy of chronic pain: assessment of consequences. Acta Anaesthesiol Scand. 1999;43 (9):909-17.
34. Sees KL, Clark HW. Opioid use in the treatment of chronic pain: assessment of addiction. J Pain Symptom Manage. 1993;8 (5):257-64.