Applied Cell Biology

Determining the Role of Wnt Signaling in the Devoplment of Neuropathic Pain; Promising Preliminary Drug Targets but Much to be Discovered

Asiye Abasian*

Department of Neurology, University of the Philippines Manila, Philipines

Corresponding author: Asiye Abasian, Department of Neurology, University of the Philippines Manila, Philipines, E-mail: asiye.abasian@gmail.com

Abstract

Wnt ligands are extracellular glycolipoproteins that bind to the frizzled family of receptors to activate the transcription co-factor β-catenin to produce a variety of downstream effects on the cell. This is becoming a larger problem due to the increasing elderly population, yet good patient outcomes are still hard to come by for clinicians. But, when mice were run through a graded mechanical stimuli test, mice injected with WNT3α had increased withdrawal reactions as opposed to vehicle-injected mice. The same dose-dependent results were found in an infrared heat plantar test. β-catenin levels in the DRG were normal suggesting that the increase in WNT in DRG neurons is related to a different signaling pathway unrelated to β-catenin after CCI. Rats given XAV939 (Wnt pathway inhibitor) intrathecally present significantly lower values for PSL-induced allodynia. More research is needed to detail the exact inflammatory pathways that Wnt signaling interacts with to create NP. This evidence shows that Wnt signaling is involved indirectly or directly in the development of CCI-induced NP via the microglial-neuron interaction and the inflammatory response.

Keywords

β-catenin
Glycolipoproteins
Neurons

ABBREVIATIONS

NP- Neuropathic Pain

CCI- Chronic Constriction Injury

TCI- Tumor Cell Implantation

FZ- Frizzled Receptor (binds Wnt ligands)

PSL- Partial Sciatic Nerve Ligation

WNT/B-CATENIN SIGNALING PATHWAY

The Wnt/β-catenin signaling pathway is one of the most studied pathways in developmental biology and development of the nervous system. Wnt ligands are extracellular glycolipoproteins that bind to the frizzled family of receptors to activate the transcription co-factor β-catenin to produce a variety of downstream effects on the cell [1]. In the absence of extracellular Wnt, cytosolic β-catenin is ubiquitinated by a destruction complex and degraded by a proteasome. When a Wnt is bound to a frizzled receptor, the destruction complex is unable to degrade β-catenin and β-catenin is transported into the nucleus where it activates the transcription factor TCF (T-cell-factor) to upregulate Wnt target genes. These genes are heavily involved embryonic and neurological development, cell proliferation, cytoskeletal remodeling, and synaptic plasticity [2].

NEUROPATHIC PAIN

Neuropathic pain (NP) is caused by damage or disease to peripheral nerves and central neurons. NP is a manifestation of neurotransmitter, molecular signaling, and ion-channel dysregulation that causes the loss of homeostatic control over nociceptive neurons that signal the somatosensory cortex [3]. NP is frequently seen in patients having diabetes mellitus, cancer and chemotherapy, HIV and associated antiviral medications, heart and stroke, peripheral nerve injury pain/neuropathy, amputation, trigeminal neuralgia, rheumatoid arthritis, neurodegenerative diseases, and other conditions. This is becoming a larger problem due to the increasing elderly population, yet good patient outcomes are still hard to come by for clinicians.

WNT3A-INDUCED PERIPHERAL NERVE NEUROPATHIC PAIN

The following provides compelling evidence for the notion that the Wnt pathway is directly involved in nociception [4]. Intraplantar injection of WNT3α to the hindpaw of wild-type mice did not elicit immediate pain in the mice. But, when mice were run through a graded mechanical stimuli test, mice injected with WNT3α had increased withdrawal reactions as opposed to vehicle-injected mice. The same dose-dependent results were found in an infrared heat plantar test. Further, when the mice were injected with inhibitors of the Wnt signaling pathway after WNT3α injection, the deleterious hyperalgesia was reverted to vehicle levels. These results suggest that WNT3α alone can cause mechanical and thermal hyperalgesia in peripheral tissues. As developed later, the cause of the WNT3α-induced NP may be related to microglial activation or an immune response via the Wnt signaling pathway.

CHRONIC CONSTRICTION INJURY (CCI) AND TUMOR CELL IMPLANTATION (TCI) UPREGULATES THE SYNTHESIS OF WNT LIGANDS IN RAT SPINAL CORD AND DORSAL ROOT GANGLIA (DRG) NEURONS

In a rat model of CCI, upregulation of WNT3α is seen in the DRG as early as 1 day after injury (via western blot). Only on day 7 did WNT3α begin to decrease to normal levels which were ultimately reached on day 21. In contrast, spinal cord levels of WNT3α were significantly increased 21 days after injury. It's possible that WNT3α is being synthesized and regulated in the spinal cord and then transported to DRG neurons due to their temporal differences in WNT3α levels. Additionally, other Wnt expression such as Wnt1, Wnt2, Wnt4, Wnt5b, and Wnt8b was elevated (consistent or systematic) in the spinal cord throughout the 21 days after injury (qRT-PCR).

Localization of the highest levels of WNT3α was found in small and medium-sized nociceptive neurons of the DRG of rats (immunofluorescence) with only moderate staining in large neurons. Small and medium-sized neurons are known to be associated with hyperalgesia (heightened sensitivity to painful stimuli) whereas large-sized neurons are known to be involved in allodynia (pain from ordinary, non-painful stimuli). In a similar study to researchers also used partial sciatic nerve ligation (PSL) in rats to induce CCI-associated NP. These researchers also found upregulation of WNT3α in the rat dorsal spinal cord of the injured side and the dorsal horn (immunochemistry and western blot). Additionally, microglial cells and other cells in the ventral horn show WNT3α staining near the nerve injury. Thus, based on the upregulation of WNT3α in response to CCI, Wnt signaling appears to have a role in NP.

Frizzled (FZ) receptors 1 and 8 (bind Wnt ligands released from the terminal bulbs of primary nociceptive fibers) on postsynaptic neurons in the spinal cord and dorsal horn were significantly up regulated 1 day after CCI in rats (immunofluorescence). Additionally, mRNA expressions (qRT-PCR) of many FZ receptor genes in the spinal cord show upregulation for up to 21 days post-CCI. FZ8 appears to be the main receptor that adjusts the receptive capacity on post-synaptic neurons as a result of the increased circulating WNTs because FZ8 shows increased nuclear transport in small and medium-sized neurons but little in large-sized neurons.

In the rat CCI group, neurons in the spinal cord

displayed an increase in nuclear β-catenin 1 day after injury showing that Wnt/β-catenin signaling is activated in the pathogenesis of nerve injury and the development of NP. In the other study, PSL also produced an increase in active β-catenin in spinal cord neurons especially on the injured side of the spinal cord. β-catenin levels in the DRG were normal suggesting that the increase in WNT in DRG neurons is related to a different signaling pathway unrelated to β-catenin after CCI.

The results of tumor cell implantation (TCI) into rat bone to recreate the NP in bone cancer patients elicited very similar results to CCI rats with regards to WNT3α and β-catenin levels. As described on the next pages, an explanation for the increase in extracellular Wnt ligands (in the absence of FZ receptor increase) in the dorsal horn and the fact that β-catenin levels in the DRG seem to be independent of extracellular Wnt is that Wnt is likely related to an associated immune response (page 4) or microglial activation (page 5) in the dorsal horn.

WNT LIGANDS ARE INVOLVED IN ACTIVATING THE IMMUNE RESPONSE TO CCI AND TCI

Based on mRNA analysis, CCI and TCI produce an increase in rat interleukin-18 and tumor necrosis factor-α transcripts which can be prevented with the WNT inhibiting drugs indicating that the WNT pathway is involved in regulating the inflammatory response which contributes to NP. These results provide support that WNT signaling has an integral role in CCI and TCI-induced NP. Determining which of these drugs is most effective in reducing NP in the rat model and obtaining optimum dosage information and more pharmacokinetics is necessary to move on to further trials. More research is needed to detail the exact inflammatory pathways that Wnt signaling interacts with to create NP.

MICROGLIAL INTERACTION WITH UPREGULATED WNT SIGNALING IN RESPONSE TO NP

There is an increasing amount of evidence that points to glia being involved in the development of neuropathic pain and nociception. Microglial cells

can detect damage via the P2 family of receptors [5]. P2 receptors bind brain-derived neurotrophic factor (BDNF) which allows pain transmitting neurons in the spinal cord to be uninhibited. BDNF levels were significantly higher in the dorsal horn of the injured side of the spinal cord in PSL rats. When recombinant WNT3α was administered to cultured MG5 cells (microglial cell from p53-KO mouse), the MG5 cells became Wnt/β-catenin pathway activated in 3 hr and BDNF levels were significantly increased (ELISA). This supports the notion that there is a glia-neuron interaction/pathway that is involved in the development of NP after PSL-induced CCI. This evidence shows that Wnt signaling is involved indirectly or directly in the development of CCI-induced NP via the microglial-neuron interaction and the inflammatory response.

POSSIBLE DRUG APPLICATIONS FOR CCI AND TCI ASSOCIATED NP

Rats given XAV939 (Wnt pathway inhibitor) intrathecally present significantly lower values for PSL-induced allodynia. When rats are given the drugs IWP-2 (inhibits WNT synthesis), Fz-8/Fc (WNT scavenger), niclosamide (inhibitor of WNT pathway), and IWR-1-endo (inhibitor of WNT pathway) intrathecally before and after CCI, mechanical allodynia is significantly reduced and the effect can be long lasting (up to 6 days for IWP-2 and Fz-8/Fc) [6]. Additionally, these same drugs can be used to reduce thermal hyperalgesia in CCI rats. Short and long-term TCI-induced NP was also significantly lowered in rats given daily IWP-2 and Fz-8 intrathecally. Stimulating the WNT signaling pathway with a WNT agonist administered intrathecally in rats produced immediate and long-lasting heightened mechanical allodynia and thermal hyperalgesia similar to that of the CCI/PSL phenotype indicating that Wnt is required in the development of NP.

The complexity of nociception after CCI seems to be understated in the literature of both the papers presented so far herein; however, both provide steps toward a solution to NP by (1) clearly identifying potential drug targets for NP (BDNF and WNT pathway inhibitors/antagonists) and (2) providing

preliminary evidence for relative effectiveness of different WNT inhibitors. Identifying all the mechanisms by which NP develops after nerve damage becomes less important once an effective drug treatment is determined and based on the experimental design of the studies herein that seems to be the ideology the authors [7] share as well.

WNT SIGNALING PATHWAY IN PATIENTS WITH HIV CAUSED NP

The first clinically associated study with regards to the interaction of Wnt signaling and NP analyzed human postmortem tissues of HIV patients with and without HIV-related NP. WNT3α was significantly upregulated in the DH of the spinal cord in 4 of the 5 patients with HIV-related NP compared to HIV negative patients (western blot). WNT 4, WNT5α, and WNT9B were also significantly upregulated when compared to HIV-positive patients without NP [8]. WNT3α staining in the superficial layers of the DH (immunoblotting) was more intense in HIV-positive patients with related NP than HIV-positive patients without NP and especially more than HIV-negative specimens. Further analysis of the DH of the spinal cord found that approximately 90% of the WNT3α staining was in NeuN (neuronal cell marker)-positive somas and extracellular spaces. WNT5α staining in HIV-positive patients with NP was scattered in the grey matter of the dorsal horn and ventral horn (approximately 95% NeuN positive) and significantly more intense than HIV-positive patients without NP. Axin2 protein (downstream target of Wnt pathway) and total β-catenin protein were significantly increased in the DH of the spinal cord in HIV-positive patients with NP when compared to HIV-positive patients without NP. These findings confirm the previous rodent studies analyzed herein and show that the Wnt/β-catenin signaling pathway is activated in HIV-related NP. Future studies regarding HIV-related NP and Wnt signaling should build on the analysis of the inflammatory mechanisms [6] and the neuron-glial interaction [7] that both contribute to the pathogenesis of NP.

CONCLUSION

WNT3α was significantly upregulated in the DH of the spinal cord in 4 of the 5 patients with HIV-related NP compared to HIV negative patients (western blot). WNT 4, WNT5α, and WNT9B were also significantly upregulated when compared to HIV-positive patients without NP. Additionally, these same drugs can be used to reduce thermal hyperalgesia in CCI rats. Short and long-term TCI-induced NP was also significantly lowered in rats given daily IWP-2 and Fz-8 intrathecally. P2 receptors bind brain-derived neurotrophic factor (BDNF) which allows pain transmitting neurons in the spinal cord to be uninhibited. BDNF levels were significantly higher in the dorsal horn of the injured side of the spinal cord in PSL rats. Further, when the mice were injected with inhibitors of the Wnt signaling pathway after WNT3α injection, the deleterious hyperalgesia was reverted to vehicle levels. These results suggest that WNT3α alone can cause mechanical and thermal hyperalgesia in peripheral tissues.

REFERENCES

1. MacDonald BT, Tamai K, He X (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 17: 9-26.

2. Rosso SB, Inestrosa NC (2013) MacDonald BT, Tamai K, He X (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 17: 9-26.

3. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH (2017) Neuropathic pain. Nat Rev Dis Primers 16: 17002.

4. Shi Y, Shu J, Gelman B, Lisinicchia JG, Tang SJ (2013) Wnt signaling in the pathogenesis of human HIV-associated pain syndromes. J Neuroimmune Pharmacol 8: 956-964.

5. Simonetti M, Agarwal N, Stösser S, Bali K, Karaulanov E, et al. (2014) Wnt-Fzd signaling sensitizes peripheral sensory neurons via distinct noncanonical pathways. Neuron 83: 104-121.

6. Trang T, Beggs S, Salter MW (2012) Brain-derived neurotrophic factor from microglia: a molecular substrate for neuropathic pain. Neuron Glia Biol 7: 99-108.

Review Article

7. Itokazu T, Hayano, Takahashi R, Yamashita T (2014) Involvement of Wnt/ β-catenin signaling in the development of neuropathic pain. Neuro Res 79: 34-40.

8. Rosso SB, Inestrosa NC (2013) WNT signaling in neuronal maturation and synaptogenesis. Front Cell Neurosci 7: 103.