ECRONICON OPEN ACCESS

EC DIABETES AND METABOLIC RESEARCH
Review Article

Molecular Mechanisms of Painful Diabetic Neuropathy

Hsinlin T Cheng*

Headache and Neuropathic Pain Unit, Department of Neurology, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, USA

***Corresponding Author**: Hsinlin T Cheng, Headache and Neuropathic Pain Unit, Department of Neurology, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts, USA.

Received: September 17, 2018; **Published:** October 31, 2018

Abstract

Painful diabetic neuropathy (PDN) is one of the most devastating symptoms from diabetic neuropathy (DN). The current available treatment for PDN has limited efficacy and is often unsatisfactory. Mechanism-specific treatment is in urgent need for treating PDN. The current review summarizes the up-to-date knowledge of the pathomechanisms of PDN. Based on information derived from animal models, potential new treatment approaches are discussed.

Keywords: *Neuropathic Pain; Diabetes Mellitus; Peripheral Neuropathy*

Introduction

Painful diabetic neuropathy (PDN), a common symptom of diabetic neuropathy, affects 40 - 50% of patients with neuropathy from type 2 diabetes, or 10 - 20% of the total diabetic population [1]. In addition, PDN could include neuropathic pain in prediabetic states as impaired glucose tolerances (IGT) or impaired fasting glucoses (IFG) before the diagnosis of diabetes [2]. According to Ziegler and colleagues, the prevalence of painful polyneuropathy is 13.3% (8.9% - 18.9%) in diabetic patients, 8.7% (2.4% - 20.0%) in subjects with IGT, 4.2% (0.9% - 11.9%) in patients with IFG, and 1.2% (0.03% - 6.7% in those with normal glucose tolerance [2]. Pain, resulting from IGT, is similar to pain which develops as a result of PDN [3]. Based on statistical analysis, 26% of patients with type 2 diabetes develop PDN, whereas only 16% of patients with type 1 diabetes develop PDN; making PDN more common in type 2 diabetes. Due to the projected increase in incidence of type 2 diabetes, PDN is believed to become a worldwide burden in the future [4].

Like other length-dependent neuropathy, PDN begins in the distal extremities and over time gradually progresses proximally. Patients with PDN often report an increase in intensity of pain at night. The pain has features of burning, tingling, electric-like, crampy, and achy sensations. In addition to the spontaneous pain, patients with PDN also develop allodynia and hyperalgesia. Allodynia is defined as normally innocuous stimulus induces painful sensations. Hyperalgesia is determined as increased sensitivity to noxious stimulation. These sensory symptoms, adding together, cause significant disability, poor quality of life, and tremendous society burden [5].

Extensive researches from animal and human studies have improved our understanding of the mechanisms underlying PDN. However, only limited treatment options are available. Of those available, many are not clearly understood and often result in adverse side effects. Further research is needed to develop and understand treatments for PDN that will provide improved efficacy. At present, treatments for PDN include topical analgesics, antidepressants, anticonvulsants, and opioids [6]. These treatments have been developed within the past 20 years, and their efficacy has been determined by double-blind, placebo-controlled clinical trials [7]. Unfortunately, only half of the patients diagnosed with PDN show signs of improvement. Furthermore, of those who show pain improvement, only a 50% approval rate has been reported [8]. Currently, it is common practice to prescribe multiple medications in order to obtain adequate control of the pain associated with PDN [9]. In addition, patients with PDN often suffer from anxiety, depression, insomnia, decreased mobility, and psychomotor impairment. Taken together, these comorbidities result in high medical costs and significantly diminish the quality of life of the patients diagnosed with PDN [10].

Molecules mediate painful diabetic neuropathy

Through the results of multiple animal studies, many suggested mechanisms have been reported for PDN. Currently, researchers believe that cell surface receptors for neurotrophic factors, ion channels, and neurotransmitters are important targets to understand the molecular pathways associated with PDN.

Sodium channels

Sodium channels, or voltage-gated (Na(v)) sodium channels, are known to be up regulated in PDN. These channels are located on nociceptive dorsal root ganglion neurons that supply Aδ or C fibers, which play key roles in the initiation and propagation of pain for the periphery to the spinal cord. It has been reported that sodium channels are up regulated during mechanical allodynia and thermal hyperalgesia. Sodium channels fall into one of two types: tetrodoxin-resistant (TTX-R) or tetrodoxin-sensitive (TTX-S). Variations in up regulation or down regulation are dependent on type of sodium channel. Craner., *et al.* reported that there are significant decreases in the expression of Na(v) 1.6 (TTX-S) and Na(v) 1.8 (TTX-R), as well as significant increases in the expression of Na(v)1.3 (TTX-S) and Na(v)1.7 (TTX-S) in diabetic rat [11]. In support of these finding, Hong and colleagues reported tyrosine phosphorylation of Na(v)1.6 and Na(v)1.7, as well as increased levels of serine/threonine phosphorylation of Na(v)1.6 and of Na(v)1.8 in diabetic dorsal root ganglia (DRG) [12]. These studies suggest that tyrosine phosphorylation of TTX-R and TTX-S sodium channels, as well as serine/threonine phosphorylation play important roles in the development of PDN. With this information, researchers believe blocking sodium channels are an effective approach for treating PDN. This hypothesis has been supported by Dick., *et al.* who reported that blocking sodium channels, blocks development of PDN in both animal models and human subjects [13]. Currently, sodium channel Nav1.7 antagonists, including Xenon 402, CNV1014802, and PF-05089771, are being tested as new therapies for PDN.

ATP receptors

Investigators have been researching the role of purines in the biochemical pathways of PDN. Adenosine triphosphate (ATP) and adenosine, both endogenous ligands, are purines that are believed to modulate pain transmission and hypersensitivity. These actions are said to occur through P1 and P2 purinoreceptors located in the peripheral and central nervous systems. P1 receptors, for adenosine, reduce inflammation and neuropathic pain by activating the A1 subtype. This has been reported in animal models and clinical studies [14]. P2 receptor involvement is believed to play a more variable role in pain processing, based on multiple subtypes [14]. For example, the P2X3 receptor has been reported to reduce pain levels and inflammation [14]. This receptor is expressed in small to medium nociceptive DRG neurons, which are IB4-postive. These neurons are also known to express transient receptor potential vanilloid receptors [14].

P2X receptors form cation-selective channels, which are permeable to sodium, potassium, and calcium [15]. Researchers have investigated the role of P2X receptors in pain processing through administration of agonists and antagonist of P2X into animal models [16]. Increase in mRNA levels of $P2X_2$ and $P2X_3$ was detected fourteen days after STZ injection in a mouse model [17]. This up regulation results in enhanced response to ATP in $P2X_3$-positive DRG neurons. PPADS and TNP-ATP, antagonists of the P2X receptors, was administered intrathecally. Mechanical allodynia was observed to be inhibited suggesting $P2X_2$, $P2X_3$, and $P2X_{(2/3)}$ are all associated with mechanical allodynia in an STZ-treated diabetic mouse model [17].

Although it is known that P2X receptors play a role in pain processing, there are no available pharmaceutical tools available to study specific P2X receptors. To create efficient pharmaceutical approaches for relief of pain, ligands with high selectivity to specific P2X receptors must be produced to study individual roles of P2X receptors in pain modulation.

TRP receptors

Transient receptor potential vanilloid 1 (TRPV1) is the most widely studied receptor studied for pain processing. Transient receptor potential receptors respond to multiple chemical and physical stimuli. TRPV1, a non-selective calcium channel, responds to capsaicin, the spicy component of hot peppers. According to a study completed by Pabbidi and colleagues, TRPV1 expression levels correlates with the levels of thermal hyperalgesia observed in STZ-treated animals [18]. Other activators of TRPV1 include glutamate, bradykinin, prostaglandins, nerve growth factor, and histamine [19]. Currently, capsaicin is offered in clinic as a topical ointment to treat PDN [7].

Nerve growth factor

Nerve growth factor (NGF), a member of the neurotrophin family, regulates the development and survival of nociceptive neurons in the central and peripheral nervous system. Most NGF actions are mediated by binding to the Trk A receptor that is expressed by the small-medium-sized nociceptive DRG neurons [20]. Thinly myelinated and unmyelinated sensory nerve fibers that project from small and medium DRG neurons are dependent on NGF during embryonic development. Postnatally, half of these nerve fibers remains dependent on NGF, and have been reported to express neuropeptides [21]. NGF has been determined to be important for the development of pain. The belief that NGF is central to nociception is supported by an animal study reported by Pezet., *et al*. who observed absence of pain sensation in an animal model that did not have NGF or Trk A [20]. Additionally, NGF has been reported to be expressed during injury in DRG neurons [20].

Further characterization of NGF has shown that it is important for the development of PDN. It has been shown in type 1 diabetes, that NGF expression increases 1.65-fold in sciatic nerve of an STZ-treated animal model [22]. Upregulation of NGF expression is believed to mediate mechanical allodynia and thermal hyperalgesia in an STZ_ model of type 1 diabetes [22]. Additionally, it has been reported that increased expression of NGF after nerve injury in DRG neurons could be the source of pain after injury [20]. With the progression of DN, NGF levels gradually decrease as a result of axonal damage, impairing NGF transport [23]. Possibly, the reduction of NGF and loss of neurotrophism could be reasoning for loss of sensation in DN [23]. Interestingly, insulin treatment of STZ-injected animals has been show to normalize nerve conduction studies, lower nociceptive molecule levels, and normalizes NGF levels and reverses conduction deficits in STZ treated animals [24].

Recently, Cheng., *et al.* studied the role of NGF in the development of mechanical allodynia in db/db mouse, a mouse model of type 2 diabetes. In db/db mouse, diabetes is developed at 4 - 5 wk of age and decreased mechanical threshold (mechanical allodynia) is present at 6 - 12 wk of age. At the later stage (< 16 wk), sensory loss follows and the mouse develops severe neuropathy [25]. They reported the increased levels of NGF and Trk A signaling in DRG neurons contributed to the development of mechanical allodynia in db/db mice. Interestingly, NGF is not only expressed by DRG neurons but also the dermal inflammatory cells during the period of PDN. The administration of a NGF antiserum reverses the pain behavior and Trk A activation, suggesting that NGF/Trk A signaling is the major contributor for PDN in db/db mice. In addition, these NGF-dependent actions are mediated by p38, an important mitogen-activated protein kinase [26]. P38 activation promotes the expression of several inflammatory molecules, such as tumor necrosis factor-α, to mediate mechanical allodynia. Taken together, NGF clearly is a major factor of PDN. Molecular cascades, which increase levels of NGF during early stages of nerve damage from diabetes results in the presence of neuropathic pain.

Recently, clinical trials using NGF neutralizing antibodies, such as tanezumab and fulnatumab, have demonstrated significant efficacy for treating PDN. Test group received tanezumab 20 mg or placebo subcutaneously on Day 1 and Week 8 reported significant pain reduction from baseline to Week 8 vs placebo group. However, no significant differences in Patient's Global Assessment of PDN were reported [27]. Fulranumab, a fully human monoclonal antibody against nerve growth factor was also tested for PDN. In a phase II, double-blind, placebo-controlled trial, PDN patients with moderate to severe PDN were randomized to treatments with fulranumab (1, 3, or 10 mg) or placebo administered subcutaneously every 4 weeks. Because of early study termination (clinical hold) by the US Food and Drug Administration, only 77 (intent-to-treat) of the planned 200 patients were enrolled. The primary endpoint, the mean reduction of average daily pain at week 12 compared with baseline, showed a positive dose-response relationship. The pair-wise comparison between the 10-mg group and placebo was significant. An exploratory responder analysis revealed that a greater proportion of patients in the 10-mg group reported ≥ 30% reduction in the average pain intensity compared with placebo at week 12. Evidence from clinical trials supported the development of future safer approaches that target NGF signaling for treating PDN.

Hepatocyte growth factor

Hepatocyte growth factor (HGF), a mesenchyme-derived cytokine with potent trophic effects on vascular and nerve tissues, has beneficial effects on PDN management. One of the most promising new gene therapy for PDN is a DNA-based therapy using a plasmid DNA that contains the human HGF gene (VM202). VM202 reduces PDN symptoms by promoting microvasculature growth and regeneration of peripheral nerves. A phase 3 study showed that PDN patients receiving 8 mg of VM202 infection per leg improved in all efficacy measures with 48.4% of the patients experienced at least a 50% reduction in mean pain score in the treated group compared with 17.6% in the placebo group after 3 months [28]. However, this analgesic effect was not statistically significant at 6 and 9 months. The study also demonstrated significant improvement in the brief pain inventory and the questionnaire portion of the Michigan Neuropathy Screening Instrument [28].

NMDA receptor

Central sensitization is described as enhances pain due to an increase in excitability of spinal cord neurons. This is the result of intracellular signaling effects activated by peripheral nerve insults. The intracellular signaling effects cause changes in neurotransmitters in the spinal cord dorsal horn (SCDH) ultimately leading to prolonged pain sensation [29].

Postsynaptic terminals in SCDH neurons are depolarized by noxious stimuli in the periphery. Thus, N-methyl-D-aspartate receptors (NMDAR) are activated in SCDH neurons. NMDAR is the receptor for glutamate [30]. When NMDAR are activated by depolarization, magnesium blockade is lifted due to calcium influx. That in turn, activates signaling cascade of proteins such as protein kinas C and mitogen

active protein kinases [31]. Inevitably, elongated depolarization causes an increase in nociceptive messages resulting in central sensitization. Although the role of NMDAR in PDN is unknown, studies have shown that MK801, an NMDAR inhibitor, and magnesium administration inhibits the development of PDN in STZ rats [32]. Dauch., *et al.* reported that increased NMDA receptor activation is important for the maintenance phase of PDN [33]. This NMDA receptor signaling not only induces downstream ERK1/2 activation but also triggers enhanced astrocytosis in the spinal cord dorsal horn [33].

Nitric oxide

Nitric oxide (NO) plays important roles in the mediation of inflammatory actions and regulated vascular perfusion to tissues. NO is known to be lipophilic, and thus can diffuse across cell membranes. It activates cyclic guanosine monophosphate (GMP)-mediated intracellular signaling. There are three known NO synthases (NOSs): neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and endothelial NOS (eNOS, NOS3). nNOS has been shown to have no role in the mediation of PDN. On the other hand, iNOS has been suggested to be a possible mediator of PDN [34]. iNOS knockout mice have been reported to show less intense DN complications, including small fiber neuropathy and nerve conduction deficits [34]. The role of eNOS in diabetes remains unknown. Studies have shown that increased NO levels in diabetes, due to advanced glycosylated and lipo-oxidation end-products could possibly contribute to the presence of tactile hyperalgesia in an STZ model of type 1 diabetes [35]. It has been reported that iNOS and nNOS are up regulated during the period of mechanical allodynia in the db/db mouse [33]. L-NAME, a nonspecific inhibitor for both NOSs, reverse mechanical allodynia and the up regulation of nNOS and iNOS in SC of db/db mouse [33].

Mitogen activated protein kinases

Activation of mitogen-activated protein kinase (MAPK) in sensory neurons in DRG and spinal cord dorsal horn (SCDH) mediate chronic pain [36]. The MAPK family consists of extracellular signal-regulated protein kinases (ERKs), p38 and c-Jun N-terminal kinase (JNK); MAPKs are associated with both inflammatory pain and neuropathic pain [37]. In an inflammatory pain model, ERKs are activated in DRG neurons and satellite cells [38]. Intrathecal administration of UO126, an inhibitor for MAPK kinase (MEK), inhibits inflammation-induced pain behavior [38]. Peripheral inflammation and axotomy also activate another member of the MAPK family, p38 [20]. Recently, Dauch., *et al.* reported that ERK activation in SCDH is essential for the maintenance of PDN in db/db mice [33]. In their report, ERK is activated in SCDH neurons of lamina I-III by a NMDA receptor-dependent mechanism.

P38 is a serine-threonine kinase which mediates cellular responses to a variety of chemical and physical insults. In a model of NGF induced hyperalgesia, p38 is phosphorylated in Trk A positive small DRG neurons and phosphorylated p38 (pp38) mediates NGF-induced up regulation of nociceptive molecules [39]. In db/db mice, a p38 inhibitor, SB203580, reverses mechanical allodynia of db/db mice and prevents the slowing of nerve conduction velocities in diabetic neuropathy, suggesting that p38 is involved in both DNP and diabetic neuropathy [40].

Like ERKs and p38, JNK has been reported to mediate PDN. Increased JNK phosphorylation was detected in DRG neurons of STZ-treated rats. Treatment of fidarestat, an aldose reductase inhibitor, reduced JNK phosphorylation and neuropathic pain. In addition, JNK is also activated in SCDH neurons by a NMDA receptor-dependent manner to mediate PDN in STZ-treated rats [41]. This phenomenon, however, was not observed in db/db mouse [33], suggesting distinct mechanisms between models of neuropathic pain for type 1 and type 2 diabetes.

Molecules that enhance glial activation

Astrocytes

Astrocyte activation (astrocytosis) is a common phenomenon in animal models of chronic pain [42]. Most of the astrocytosis in chronic painful states is in SCDH. This activation is demonstrated by increased glial acid fibrillary protein (GFAP) expression, cell size and length of cell processes. The functions of activated astrocytes were summarized by Chiang., *et al* [42]. Briefly, the activated astrocytes release several activating factors to enhance the activity of secondary sensory neurons in SCDH. These factors include glutamate, STP, D-serine, neurotrophic factors, cytokine and chemokines. These glio-transmitters interact with cell surface receptors on nearby neurons, astrocytes, and microglia and trigger a network of neuron-glial interactions. In db/db mice, astrocyte activation in SCDH was reported during the period of mechanical allodynia via NMDA receptor-mediated mechanisms [33]. In these reports, these neuron-astrocyte interactions involve interleukin IL-1β and NOSs [33].

Microglia

Microglial activation in SCDH is a common phenomenon in most of the animal models of chronic pain, including PDN [43]. In STZ treated rodents, microglia activation is detected in SCDH. This activation is regulated by NMDA receptor and ERK [44]. The activated microglia

express increased levels of microglial markers, including Iba1 and OX-42. In addition, they also have increased numbers, hypertrophic morphology, and thick processes. Furthermore, activated microglia express activated ERK and Src-family kinase (SFK) [45].

Developing mechanism-specific treatments

At present, great efforts are being made to improve management of PDN. Per the published evidence-based placebo-controlled clinical trials, guidelines for the treatment of PDN have recently been created. The 2006 and 2010 guidelines from the European Federation of Neurological Societies task Force (EFNS) [46] and the 2011 guidelines from the American Academy of Neurology (AAN), the American Association of Neuromuscular and Electrodiagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation [47] are the most thorough and recent guidelines on this topic. However, as described in most guidelines, most published studies use 50% pain relieve as an indicator for a success trial, that is far from ideal for patients to improve their life quality. Based on the current review, several new approaches could be potential promising directions to create effective treatments for PDN.

To improve our understanding of the mechanisms underlying PDN, research through animal studies and clinical trials must be accomplished. Through these studies, we can advance our understanding of the pathogenesis of PDN, which would provide opportunity to create new treatments specific for this highly morbid health problem. These mechanism-specific therapies would focus on genes, proteins, and signaling cascades important for the development of PDN. Furthering our understanding of these pathways would be beneficial for the management of the devastating complication of diabetes.

It is vitally important that more studies are conducted to further the understanding the mechanisms associated with PDN. Because of animal studies and clinical trials, researchers have could develop treatments for PDN. However, currently it is common practice for clinicians to prescribe multiple medications to be taken at one time for patients to obtain adequate relief of pain. However, complete understanding of some available medications has yet to be obtained. Likewise, often, adverse consequences result from treatments specific for PDN. In the future, we must continue efforts to enhance the understanding of the biochemical pathways underlying PDN through animal model studies and clinical trials. Scientists have been awarded for many breakthroughs in understanding PDN through animal studies. However, due to variation in human subjects, robust results observed in animal studies does not always correlate with what results in human subjects. In the end, further studies are needed to understand the complex mechanism of PDN to enhance medical management for the complications of both type 1 and type 2 diabetes.

Conclusions

Mechanism-specific treatments are in urgent need for treating PDN. As summarized in this review, several potential molecules have been identified in animal research that could be key factors of PDN. Clinical trials that use specific antagonists or gene therapies that down regulate these target molecules are the right strategies for developing effective treatments for PDN.

Bibliography

1. Galer BS., *et al*. "Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life". *Diabetes Research and Clinical Practice* 47.2 (2000): 123-128.

2. Ziegler D., *et al*. "Neuropathic pain in diabetes, prediabetes and normal glucose tolerance: the MONICA/KORA Augsburg Surveys S2 and S3". *Pain Medicine* 10.2 (2009): 393-400.

3. Singleton JR., *et al*. "Polyneuropathy with Impaired Glucose Tolerance: Implications for Diagnosis and Therapy". *Current Treatment Options in Neurology* 7.1 (2005): 33-42.

4. Dworkin RH., *et al*. "Healthcare utilization in people with postherpetic neuralgia and painful diabetic peripheral neuropathy". *Journal of the American Geriatrics Society* 59.5 (2011): 827-836.

5. Jensen MP., *et al*. "Do pain qualities and spatial characteristics make independent contributions to interference with physical and emotional functioning?" *Journal of Pain* 7.9 (2006): 644-653.

6. Ziegler D. "Treatment of diabetic polyneuropathy: Update 2006". *Annals of the New York Academy of Sciences* 1084 (2006): 250-266.

7. Edwards JL., *et al*. "Diabetic neuropathy: mechanisms to management". *Pharmacology and Therapeutics* 120.1 (2008): 1-34.

8. Jensen TS., *et al*. "New perspectives on the management of diabetic peripheral neuropathic pain". *Diabetes and Vascular Disease Research* 3.2 (2006): 108-119.

9. Gilron I., *et al*. "Nortriptyline and gabapentin, alone and in combination for neuropathic pain: a double-blind, randomised controlled crossover trial". *Lancet* 374.9697 (2009): 1252-1261.

10. Dworkin RH., *et al*. "Impact of postherpetic neuralgia and painful diabetic peripheral neuropathy on health care costs". *Journal of Pain* 11.4 (2010): 360-368.

11. Craner MJ., *et al*. "Changes of sodium channel expression in experimental painful diabetic neuropathy". *Annals of Neurology* 52.6 (2002): 786-792.

12. Hong S., *et al*. "Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat". *Journal of Biological Chemistry* 279.28 (2004): 29341-29350.

13. Dick IE., *et al*. "Sodium channel blockade may contribute to the analgesic efficacy of antidepressants". *Journal of Pain* 8.4 (2007): 315-324.

14. Liu XJ and MW Salter. "Purines and pain mechanisms: recent developments". *Current Opinion in Investigational Drugs* 6.1 (2005): 65-75.

15. Jacobson KA., *et al*. "Purine and pyrimidine (P2) receptors as drug targets". *Journal of Medicinal Chemistry* 45.19 (2002): 4057-4093.

16. Tsuda M., *et al*. "Mechanical allodynia caused by intraplantar injection of P2X receptor agonist in rats: involvement of heteromeric P2X2/3 receptor signaling in capsaicin-insensitive primary afferent neurons". *Journal of Neuroscience* 20.15 (2000): RC90.

17. Migita K., *et al*. "Modulation of P2X receptors in dorsal root ganglion neurons of streptozotocin-induced diabetic neuropathy". *Neuroscience Letters* 452.2 (2009): 200-203.

18. Pabbidi RM., *et al*. "Influence of TRPV1 on diabetes-induced alterations in thermal pain sensitivity". *Molecular Pain* 4 (2008): 9.

19. Cesare and McNaughton. "A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin". *Proceedings of the National Academy of Sciences of the United States of America* 93.26 (1996): 15435-15439.

20. Pezet S and SB McMahon. "Neurotrophins: mediators and modulators of pain". *Annual Review of Neuroscience* 29 (2006): 507-538.

21. McMahon SB. "NGF as a mediator of inflammatory pain". *Philosophical transactions of the Royal Society of London. Series B: Biological Sciences* 351.1338 (1996): 431-440.

22. Fernyhough P., *et al*. "Altered neurotrophin mRNA levels in peripheral nerve and skeletal muscle of experimentally diabetic rats". *Journal of Neurochemistry* 64.3 (1995): 1231-1237.

23. Tomlinson DR., *et al*. "Neurotrophins and peripheral neuropathy". *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 351.1338 (1996): 455-462.

24. Biessels GJ., *et al*. "Insulin partially reverses deficits in peripheral nerve blood flow and conduction in experimental diabetes". *Journal of the Neurological Sciences* 140.1-2 (1996): 12-20.

25. Cheng HT., *et al*. "Nerve growth factor mediates mechanical allodynia in a mouse model of type 2 diabetes". *Journal of Neuropathology and Experimental Neurology* 68.11 (2009): 1229-1243.

26. Cheng HT., *et al*. "Nerve growth factor/p38 signaling increases intraepidermal nerve fiber densities in painful neuropathy of type 2 diabetes". *Neurobiology of Disease* 45.1 (2011): 280-287.

27. Bramson C., *et al*. "Exploring the role of tanezumab as a novel treatment for the relief of neuropathic pain". *Pain Medicine* 16.6 (2015): 1163-1176.

28. Kessler JA., *et al*. "Double-blind, placebo-controlled study of HGF gene therapy in diabetic neuropathy". *Annals of Clinical and Translational Neurology* 2.5 (2015): 465-478.

29. Woolf CJ. "Central sensitization: uncovering the relation between pain and plasticity". *Anesthesiology* 106.4 (2007): 864-867.

30. Costigan M and CJ Woolf. "Pain: molecular mechanisms". *Journal of Pain* 1.3 (2000): 35-44.

31. Cheng HT., *et al*. "Inflammatory pain-induced signaling events following a conditional deletion of the N-methyl-D-aspartate receptor in spinal cord dorsal horn". *Neuroscience* 155.3 (2008): 948-958.

32. Begon S., *et al*. "Magnesium and MK-801 have a similar effect in two experimental models of neuropathic pain". *Brain Research* 887.2 (2000): 436-439.

33. Dauch JR., *et al*. "Neuron-astrocyte signaling network in spinal cord dorsal horn mediates painful neuropathy of type 2 diabetes". *Glia* 60.9 (2012): 1301-1315.

34. Vareniuk I., *et al*. "Inducible nitric oxide synthase gene deficiency counteracts multiple manifestations of peripheral neuropathy in a streptozotocin-induced mouse model of diabetes". *Diabetologia* 51.11 (2008): 2126-2133.

35. Khan N., *et al*. "Ameliorative potential of spironolactone in diabetes induced hyperalgesia in mice". *Yakugaku Zasshi* 129.5 (2009): 593-599.

36. Ji RR., *et al*. "MAP kinase and pain". *Brain Research Reviews* 60.1 (2009): 135-148.

37. Sweitzer SM., *et al*. "Antinociceptive action of a p38alpha MAPK inhibitor, SD-282, in a diabetic neuropathy model". *Pain* 109.3 (2004): 409-419.

38. Obata K., *et al*. "Activation of extracellular signal-regulated protein kinase in the dorsal root ganglion following inflammation near the nerve cell body". *Neuroscience* 126.4 (2004): 1011-1021.

39. Ji RR., *et al*. "p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia". *Neuron* 36.1 (2002): 57-68.

40. Cheng HT., *et al*. "p38 mediates mechanical allodynia in a mouse model of type 2 diabetes". *Molecular Pain* 6 (2010): 28.

41. Daulhac L., *et al*. "Diabetes-induced mechanical hyperalgesia involves spinal mitogen-activated protein kinase activation in neurons and microglia via N-methyl-D-aspartate-dependent mechanisms". *Molecular Pharmacology* 70.4 (2006): 1246-1254.

42. Chiang CY., *et al*. "Role of Astrocytes in Pain". *Neurochemical Research* 37.11 (2012): 2419-2431.

43. Hulsebosch CE. "Special issue on microglia and chronic pain". *Experimental Neurology* 234.2 (2012): 253-254.

44. Daulhac L., *et al*. "Phosphorylation of spinal N-methyl-d-aspartate receptor NR1 subunits by extracellular signal-regulated kinase in dorsal horn neurons and microglia contributes to diabetes-induced painful neuropathy". *European Journal of Pain* 15.2 (2011): 169. e1-169.e12.

45. Tsuda M., *et al*. "Activation of dorsal horn microglia contributes to diabetes-induced tactile allodynia via extracellular signal-regulated protein kinase signaling". *Glia* 56.4 (2008): 378-386.

46. Attal N., *et al*. "EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision". *European Journal of Neurology* 17.9 (2010): 1113-e88.

47. Bril V., *et al*. "Evidence-based guideline: Treatment of painful diabetic neuropathy: report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation". *Neurology* 76.20 (2011): 1758-1765.

Volume 2 Issue 2 November 2018