CHAPTER 1: Introduction and Literature Review

1.1 Introduction

Cancer remains a significant global health challenge, contributing substantially to leading cause of death and presenting intricate hurdles for healthcare systems and societies across the globe (Islami et al., 2024). One of the most widely used approach for the treatment of cancer is chemotherapy, which acts through multifarious pathways to achieve therapeutic effectiveness. Unfortunately, a major drawback of chemotherapy lies in its substantial toxicities, primarily stemming from unintended off-target effects (Polomano et al., 2023). Chemotherapy-induced neuropathic pain (CINP) is one of the major clinical toxicity associated with the use of chemotherapeutic agents such as platinum compounds, vinca alkaloids and taxanes. CINP results from the damage or dysfunction of peripheral nerves, nerve roots, or the central nervous system caused by exposure to chemotherapeutics agents. Unlike nociceptive pain, which arises from the activation of pain receptors in response to tissue damage, neuropathic pain emerges from aberrant neural signalling and processing. Patients often describe CINP symptoms burning, shooting, tingling, or electric shocks, highlighting its unique sensory qualities (Banach et al., 2017).

The prevalence of CINP is notably high as it occurs in almost 68.1% of the patients receiving chemotherapy. The platinum-based antineoplastic drugs upsurge the prevalence of CINP and mortality with high doses i.e. 250-350 mg/m^2 of cisplatin and 500-600 mg/m^2 of oxaliplatin (Burgess et al., 2021; Staff et al., 2017). Paclitaxel is slightly more neurotoxic than docetaxel, however, the neurotoxic threshold for paclitaxel is 400 mg/m^2 and for docetaxel is around 1000 mg/ m^2 (Velasco and Bruna,

2015). Vincristine is the another antineoplastic agent with strong potential causing neuropathic pain in patients at the dosage of 4 mg/m^2. Even the continuous use of general chemotherapeutic agents at the lower dose of 6-8 mg/m^2 can cause the same level of distress (Chiba et al., 2017a). Thus, CINP is an inevitable condition accompanied by chemotherapy in cancer patients. Higher doses of chemotherapeutics contribute to the effective treatment of cancer, at the same time resulting in CINP. Although lower doses have comparatively lesser risk but chronic treatment with low doses may leads to severe CINP condition.

CINP can impact the sensory system in a pattern reminiscent of a "stocking and glove," resulting in the onset of symptoms such as allodynia, hyperalgesia, numbness, tingling, paresthesia, and gait disturbances (Starobova and Vetter, 2022). This condition has the potential to impair various functional abilities and significantly diminish the overall quality of life for patients, often leading to the necessity for dose reduction, treatment discontinuation and compromising the survival prospects of patients. It's noteworthy that many therapeutics available for managing neuropathic pain are often ineffective in the case of CINP patients due to the distinct cellular and molecular etiologies associated with both conditions (Hou et al., 2018; Sisignano et al., 2014). Chemotherapy leads to development of peripheral neuropathy by striking the somatosensory nervous system, increasing the neuronal firings and activation of nociceptive mediators including transient receptor potential channels (TRPs) and other ion channels (Kawashiri et al., 2021). The pathophysiological contributors in the progression and development of the CINP was depicted in the figure 1.1.

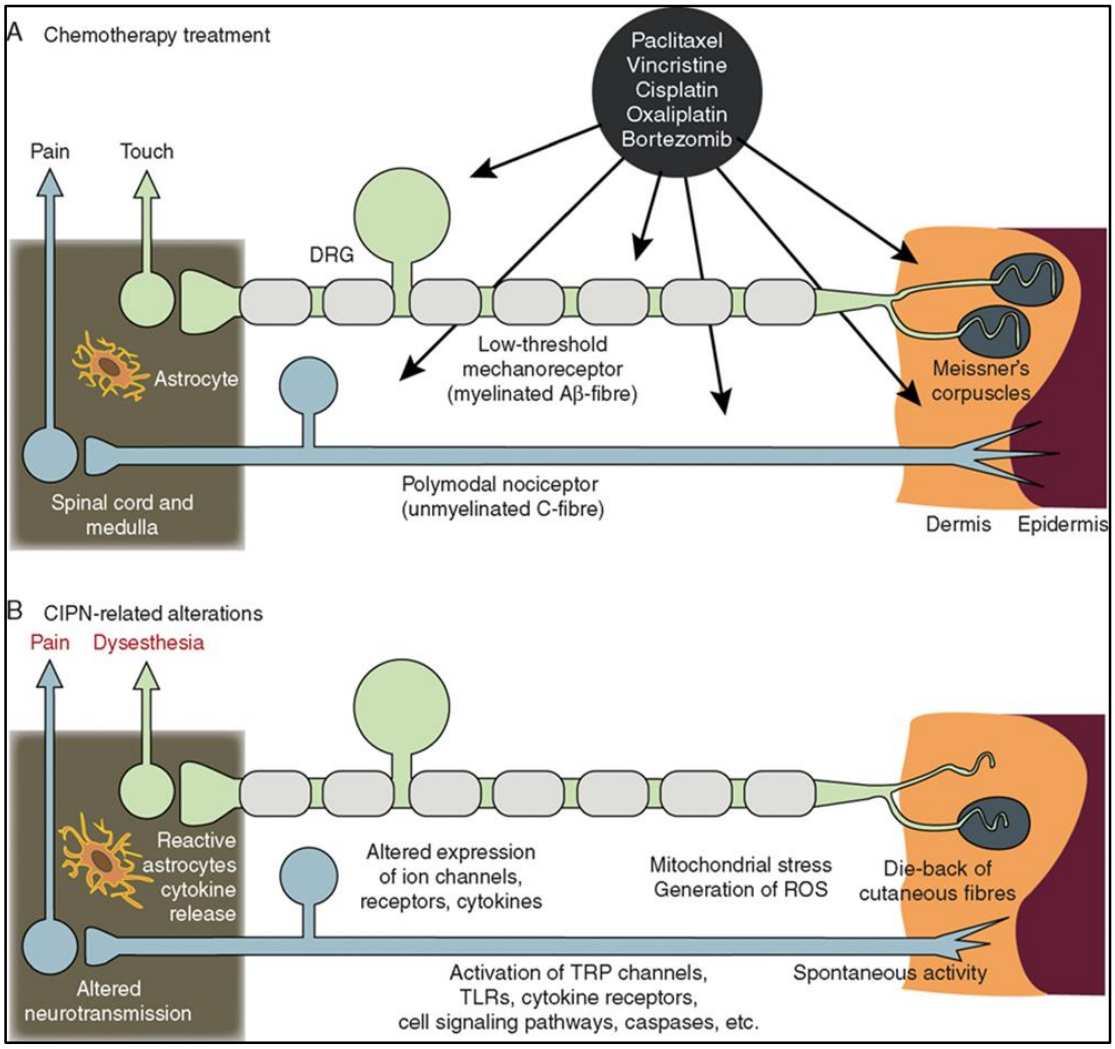

Figure 1.1 Pathophysiological events contributing to CINP. **A)** the most common agents triggering CIPN are depicted in bubbles, with arrows indicating their respective sites of action. The labeled structures in Figure A are detailed in Figure B, summarizing the changes occurring in these structures during the development of CIPN. The information presented is sourced from Boyette-Davis JA, Walters ET, Dougherty PM's article "Mechanisms involved in the development of chemotherapy-induced peripheral neuropathy," published in Pain Management (2015) (Cheng et al., 2015).

1.2 Understanding the mechanisms underlying chemotherapy-induced neuropathic pain

1.2.1 Vincristine-induced peripheral neuropathy

Vincristine, a potent cytostatic alkaloid originally derived from the Madagascar periwinkle (*Catharanthus roseus*), stands out among vinca alkaloids for its robust

neurotoxic effects (Banach et al., 2017). The susceptibility to CINP following vincristine treatment has been associated with specific single nucleotide polymorphisms (SNPs). Notably, these SNPs impact genes related to crucial inflammatory signaling pathways (PPARD, LTA), transporters (ABCC4, ABCC5, SLC10A2), and enzymes involved in oxidative metabolism (ALDH1A1, GLI1) (Fernandez-Lizarbe et al., 2013; Hu et al., 2017; Susa et al., 2010). Similar to paclitaxel, vincristine disrupts axonal transport by stabilizing microtubules, leading to axonal degradation and axonopathy (Boyette-Davis et al., 2015). Vincristine treatment also enhances the activity of T-type calcium channels and TRP channels in sensory neurons of the DRG (Chiba et al., 2017a). Oxidative stress and inflammation contribute to vincristine-induced pain, mirroring the mechanisms observed with paclitaxel. Activation of nitric oxide synthase (NOS) and the cyclic guanosine monophosphate (cGMP) pathway increases nocifensive behavior after vincristine treatment, and this effect can be reversed by NOS inhibitors. Vincristine triggers an inflammatory response in the peripheral nervous system (PNS), leading to the recruitment of monocytes expressing CX3C chemokine receptor 1 (CX3CR1) to the sciatic nerve, resulting in reactive oxygen species (ROS) production that activates TRPA1/TRPV1 (El-Masry et al., 2012; Sánchez et al., 2023). In the rat spinal cord, vincristine treatment induces significant activation of astrocytes, leading to the release of IL-1β and phosphorylation of NMDAR. The multifaceted impact of vincristine on neuronal and inflammatory pathways contributes to the intricate pathogenesis of vincristine-induced neuropathic pain (Figure 1.2) (Canta et al., 2015; Flatters and Bennett, 2004).

1.2.2 Cisplatin-induced neuropathic pain

Cisplatin, a platinum derivative extensively utilized for the treatment of colorectal carcinomas, introduces distinct neuropathic symptoms, encompassing an initial acute pain syndrome reminiscent of P-APS and subsequent chronic distal sensory neuropathy (Kuai et al., 2020a). These effects deviate from the mechanisms observed with paclitaxel and vincristine, with cisplatin exhibiting heightened impacts on neurons, particularly in the realm of ligand-gated and voltage-gated ion channels. Internally, cisplatin undergoes degradation to derivatives such as oxalate and platinum (Naziro*f*ûLu, 2018). Oxalate, through chelation of intracellular Ca^{2}+, disrupts neuronal membrane potential and modulates the activity of voltage-gated ion channels. Various TRP channels, including TRPA1, TRPV1 mediating mechanical and cold allodynia, and sensitization of TRPV1, TRPM8, and TRPV4 in dorsal root ganglion (DRG) neurons, contribute to cisplatin induced pain (Naziroğlu and Braidy, 2017a). Cisplatin also induces cooling-induced bursts of action potentials in myelinated A fiber through sodium channel subtype NaV1.6. Similar to paclitaxel and vincristine, cisplatin induces cellular damage, possibly through the activation of the p38–mitogen-activated protein kinase (MAPK) pathway linked to DNA damage recognition by high-mobility group box 1 in DRG neurons (Kuai et al., 2020b). Oxidative stress and inflammation contribute to cisplatin induced neuropathic pain, with reactive oxygen species (ROS) production in isolectin IB4-binding DRG and spinal neurons, potentially in response to mitochondrial dysfunction (Sakallı Çetin et al., 2017; Sharawy et al., 2015). Cisplatin-induced neuropathic pain also involves activation of NMDARs and NOS in the spinal cord, along with increased activation of satellite glial cells and astrocytic gap junctions.

However, this astrocyte activation appears less severe than the microgliosis observed with paclitaxel or vincristine and subsides 14 days' post-initial treatment (Figure 1.2).

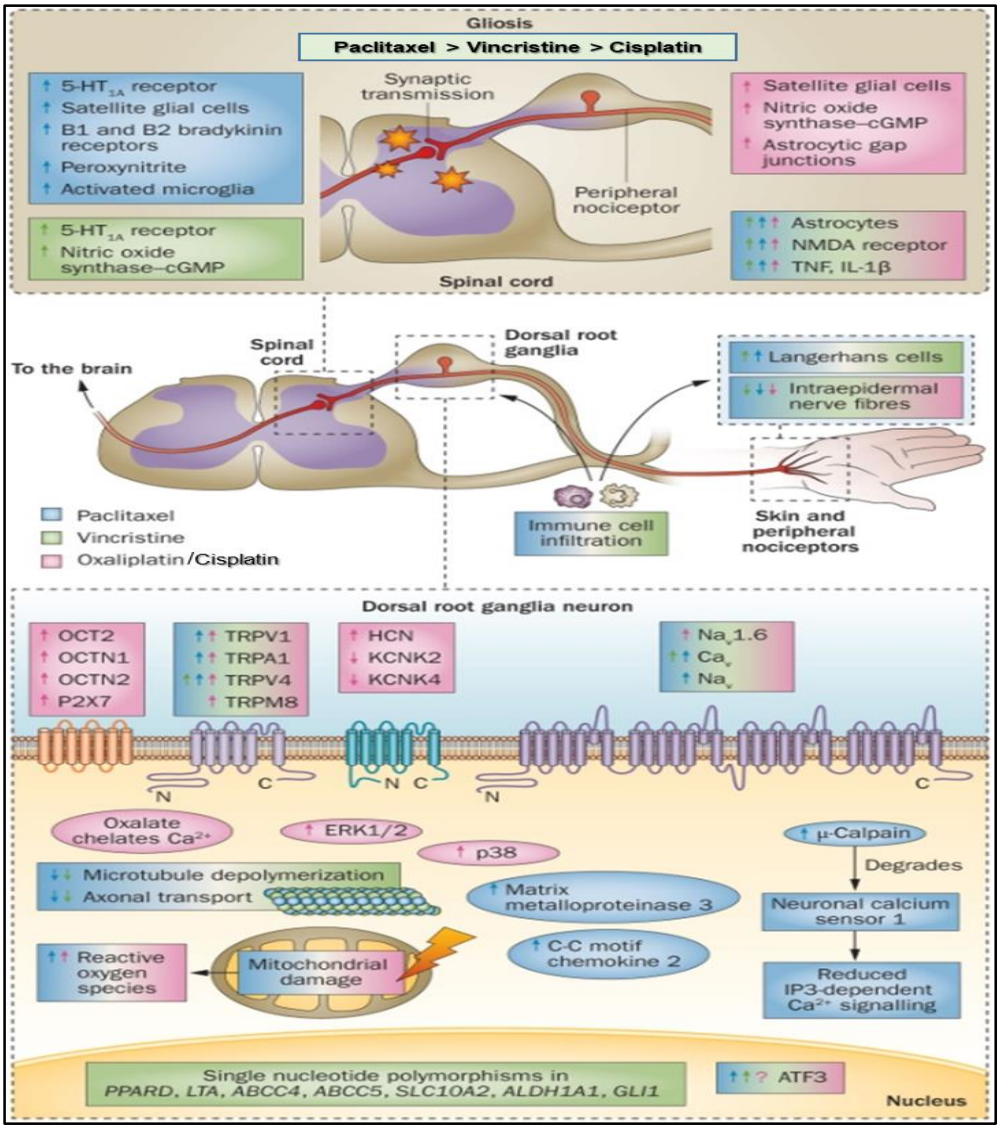

Figure 1.2 Illustrates the mechanisms of chemotherapy-induced peripheral neuropathic pain: Highlighting the pathophysiological alterations triggered by paclitaxel (depicted in blue), vincristine (in green), and oxaliplatin/cisplatin (in pink) within the spinal cord, dorsal root ganglia, peripheral nervous system (PNS), and skin. The abbreviations used include 5-HT (5-hydroxytryptamine), ATF3 (cyclic AMP-dependent transcription factor 3), Cav (voltage-gated calcium channel), cGMP (cyclic GMP), ERK1/2 (mitogen-activated protein kinases), HCN (potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel), IP3 (inositol 1,4,5-trisphosphate), KCNK (potassium channel, subfamily K), Nav (voltage-gated sodium channel), NMDA (N-methyl-d-aspartate), OCT2 (solute carrier family 22 member 2-organic cation transporter), OCTN 1 and 2 (solute carrier family 22 members 4 and 5 -

organic cation/carnitine transporters), P2X7 (P2X purinoceptor 7), TNF (tumour necrosis factor), and TRP (transient receptor potential cation channel). Reprinted with permission by springer nature from source reference (Podratz et al., 2011).

1.2.3 Peripheral sensitization

Peripheral sensitization plays a crucial role in CINP, contributing to the amplification and maintenance of pain signals. Peripheral sensitization is the increased responsiveness of nociceptive neurons in the periphery due to the continuous presence of a stimulus and several signaling pathways are associated with this phenomenon (Costigan and Woolf, 2000; Wang et al., 2006). After tissue injury release of different mediators occurs at the site including bradykinin, nerve growth factors, adenosine tri-phosphate, histamine, interleukins, etc. These mediators stimulate the ion channels present on the nociceptive terminal by direct (phosphorylation) or indirect mechanisms (prostaglandin pathway) (Fornasari, 2012; Wainger and Brenner, 2017; Wang et al., 2006). Stimulation of nociceptors initiates the peripheral sensitization often accompanied by cytokines storm, protein phosphorylation, and ion channel activation which in chronic terms modify the gene transcription (Basbaum et al., 2009; Fornasari, 2012; Price et al., 2018; Wainger and Brenner, 2017). Moreover, the sensitization of peripheral nerve endings can lead to an exaggerated release of neurotransmitters, such as substance P and glutamate, at the synapses between peripheral nerves and dorsal root ganglion neurons. This enhanced neurotransmitter release contributes to the transmission of pain signals to the spinal cord and the central nervous system.

Activation of G protein-coupled receptors (GPCR) or serine/threonine kinases such as protein kinase A and protein kinase C occur which further activate the downstream signaling and modulate cellular activity especially the electric impulse

across the PNS. The vicious cycle of inflammation and nociceptor activation gets developed due to continuous stimulation of ion channels (e.g., TRPV1, $Na_V1.9$) and the release of inflammatory mediators (Arribas-Blázquez et al., 2019; Berta et al., 2017; Mickle et al., 2016). Another mechanism of peripheral sensitization is altered intracellular signaling and change in substrates for activation which is independent of altered nociceptive threshold and relies upon the heightened sensitivity and cross-interactions of pathways inside the cell (Figure 1.3).

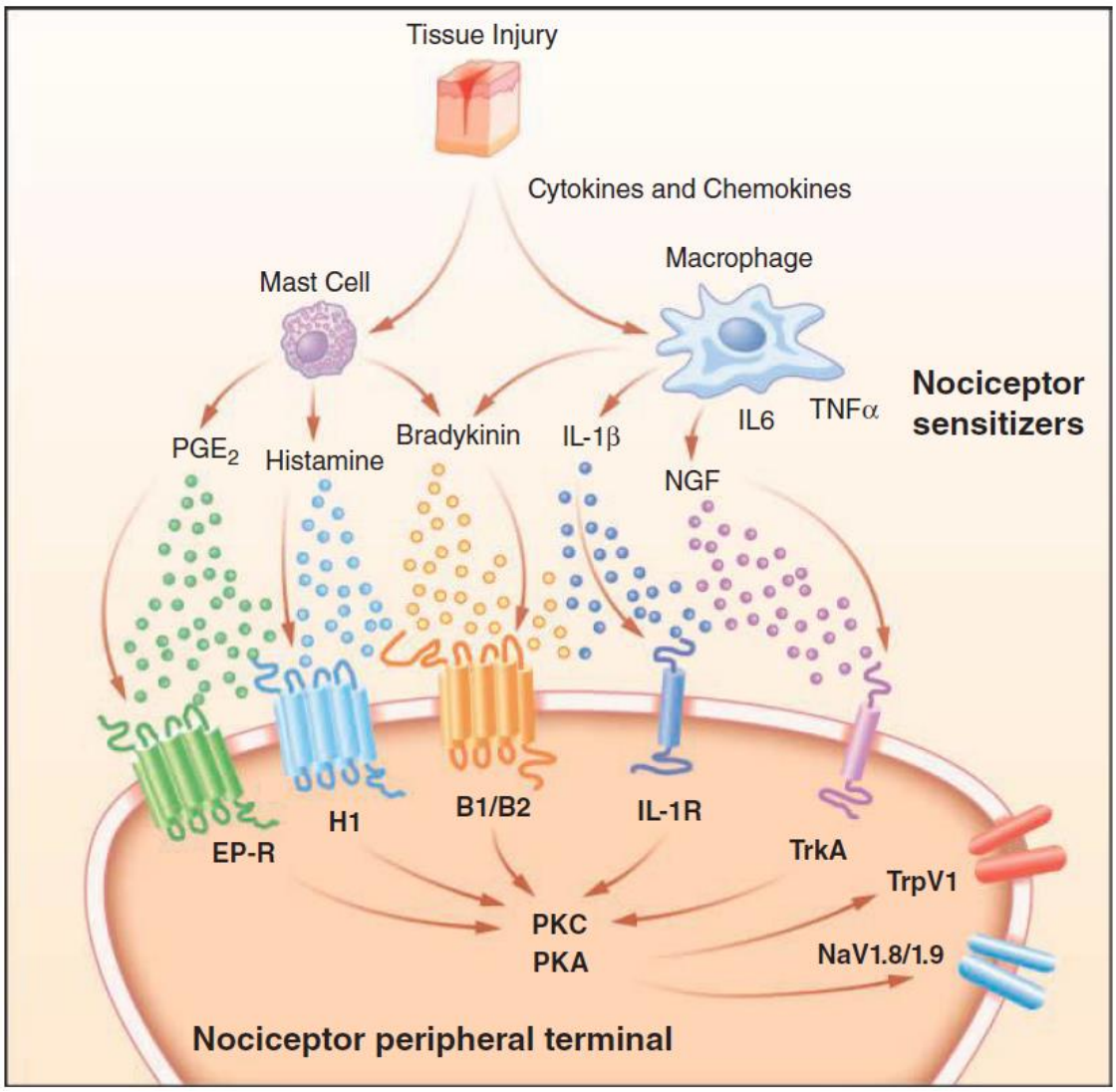

Figure 1.3 Peripheral sensitization under CINP. Reprinted with permission by McGraw Hill LLC from source reference (Wainger and Brenner, 2017).

1.2.4 Central sensitization

Central sensitization is a phenomenon that occurs in spinal cord and brain regions and increases pain responsiveness (Ji et al., 2018; Woolf, 2007). This feature of CINP is very unique as, unlike peripheral sensitization, it can persist without the presence of the stimulus and is adequate independently to develop hypersensitivities (Wainger and Brenner, 2017). In presynaptic sites the nociceptor terminal activation occurs that result in the initiation of ERK and p38 MAPK pathway which in turn facilitate the release of glutamate into the synaptic cleft (Ji et al., 2018). These series of events activate NMDA and AMPA receptors with simultaneous inhibition of potassium channels (e.g., Kv4.2 and Kv1.2), which further mediates several downstream pathways thereby inducing the nociceptive gene expression (Wainger and Brenner, 2017; Zhao et al., 2013). Temporal windup or summation are the terms used to define the central sensitization where repeated stimulation led to persistent pain even after the removal of stimuli. Glutamate and its receptors N-methyl-d-Aspartate (NMDA) participate in the development and maintenance of central sensitization which is Ca^{++} impermeable under normal conditions but due to certain mediators (e.g., Nerve injury associated molecules) the Mg+ block is removed and calcium enters the cell causing the neuronal excitability (Uniyal et al., 2021b). The process is maintained by activity of various enzymes such as MAPK, PKA, PKC extracellular signal-related kinase (ERK) and Src (Ji et al., 2018). Ion channels such as Kv4.2 also promote the NMDA induced excitotoxicity as the ERK inhibits these channels which resist the neuronal homeostasis. Apart from this, different cytokines, prostaglandin, BDNF, substance P also excite NMDA receptors and promotes thermal and mechanical hypersensitivity. Microglia, astrocytes, axonal degeneration, immune cell infiltration, protein phosphorylation and enhanced

intracellular trafficking are other key mediators of central sensitization (Figure 1.4) (Ji et al., 2018; Wood, 2020; Woolf, 2007).

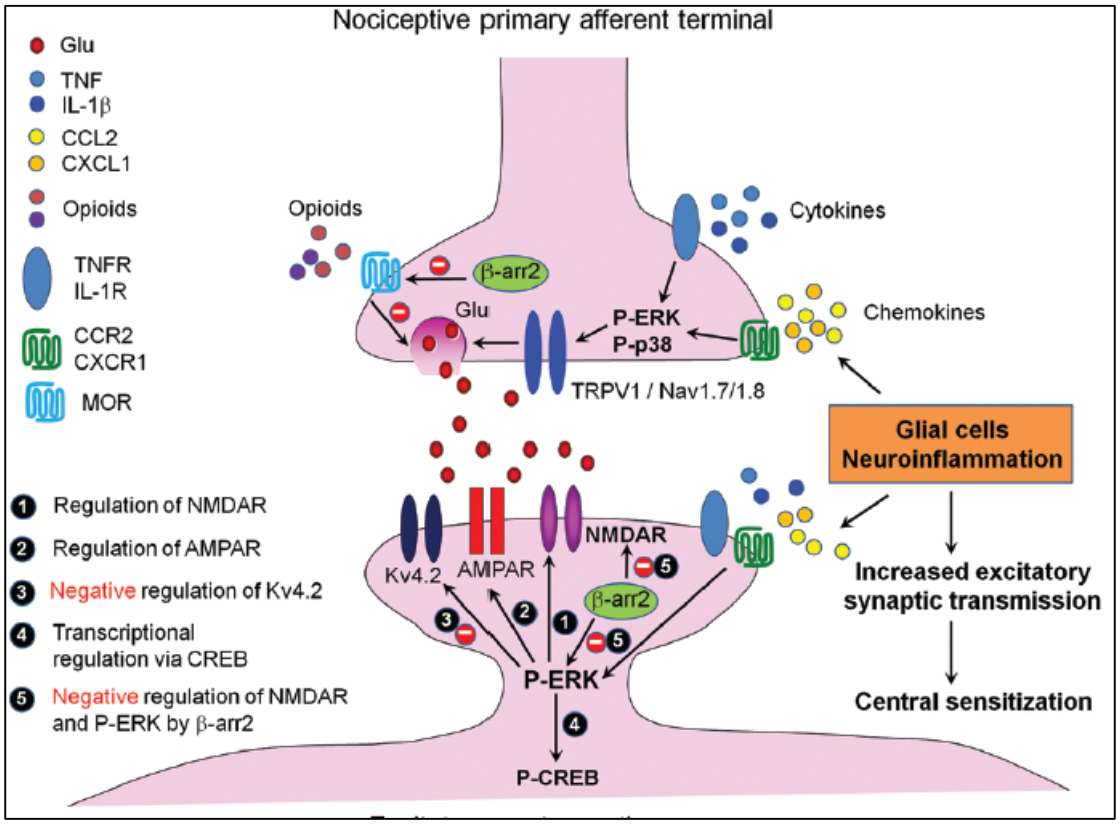

Figure 1.4 Molecular mechanism of central sensitization in CINP. Reprinted or adopted with permission by American Society of Anesthesiologists from source reference (Ji et al., 2018).

1.2.5 Neuronal Damage and Axonal Dysfunction

Chemotherapeutic agents such as platinum compounds and taxanes, have been implicated in causing direct damage to peripheral nerves. This neuronal injury disrupts axonal integrity, leading to aberrant signaling and the initiation of neuropathic pain pathways. Ultimately, axonal degeneration pathways can be triggered by various events. Chemotherapy-induced axonal degeneration may arise from various potential mechanisms that trigger axonal damage specifically related to some properties of each drug class: altered axonal transport, modified mitochondrial functioning, changes in ion

channels and calcium homeostasis, neuroinflammation and DNA damage. Several chemotherapy classes disrupt microtubule functioning, thereby arresting cancer cells in metaphase and inducing cell death. These classes include taxanes, epothilones, eribulin, and vinca alkaloids. Additionally, the neurotoxicity of vedotins is likely due to their potent antitubulin activity. Taxanes and epothilones hyperstabilize microtubules, preventing microtubule depolymerization. Conversely, vinca alkaloids and vedotins promote microtubule depolymerization. Eribulin interferes with microtubule dynamics by predominantly binding to high-affinity sites at the plus ends of existing microtubules. The disruption of microtubule dynamics by typical antitubulin agents and proteasome inhibitors may negatively affect both anterograde and retrograde axonal transport. In accordance with this, several in vitro studies have demonstrated that eribulin, vincristine, paclitaxel, and ixabepilone inhibit both anterograde and retrograde transport (Araldi et al., 2024; Chen et al., 2024).

1.2.6 Oxidative Stress, Mitochondrial Dysregulation and Inflammation

Chemotherapy-induced oxidative stress and inflammation play a pivotal role in the pathogenesis of CINP. Chemotherapeutic agents generate reactive oxygen species (ROS) within peripheral nerves, causing oxidative damage to lipids, proteins, and DNA. This damage triggers the release of danger-associated molecular patterns (DAMPs), activating immune cells and leading to the release of pro-inflammatory cytokines. The resulting neuroinflammation sensitizes nerve endings, contributing to the generation of pain signals. Oxidative stress not only compromises mitochondrial function but also activates transcription factors like NF-κB and AP-1, regulating inflammatory gene expression. This sustained activation contributes to chronic inflammation observed in CINP (Schiavone and Trabace, 2017). Additionally, oxidative stress and inflammation induce peripheral and central sensitization, amplifying pain signals and contributing to

allodynia and hyperalgesia. The imbalance between oxidative stress and antioxidant defenses further contributes to nerve damage. Understanding this intricate relationship provides a foundation for developing targeted therapies, including antioxidant and anti-inflammatory interventions, with the potential to alleviate chemotherapy-induced neuropathic pain and enhance the overall well-being of cancer patients undergoing treatment. Mitochondrial damage is another significant contributor to paclitaxel-induced neuropathic pain. Paclitaxel induces mitochondrial swelling and alters mitochondrial morphology in C fibers and myelinated axons, possibly by opening the mitochondrial permeability transition pore. This pore opening leads to Ca^{2+} efflux from mitochondria, as observed in rat models of paclitaxel-induced CINP. *In-vitro* studies on rat sciatic nerves have further demonstrated that paclitaxel treatment diminishes the activity of mitochondrial respiratory chain complexes I and II (Canta et al., 2015).

1.2.7 Increase ion channel activity

Enhanced ion channel activity has been implicated in the development of paclitaxel induced neuropathy which is associated with the sensitization of peripheral sensory neurons (Baker et al., 2023). Although paclitaxel itself does not directly activate ion channels, it is believed that endogenous signaling mediators and cellular pathways contribute to the sensitization observed in CINP. Phosphorylation of N-methyl-D-aspartate receptors (NMDARs) and the subsequent increase in their activation at postsynaptic terminals are thought to play a role in the development of PINP (Xie et al., 2017). In rodent models of CINP, paclitaxel treatment has been found to enhance the activity of both ligand-gated and voltage-gated ion channels. The use of tetrodotoxin, an inhibitor of voltage-gated cation channels, reduced neuropathic pain in mice, emphasizing the involvement of voltage-gated ion channels in paclitaxel-induced

PINP (Salas et al., 2015). Additionally, TRP channels, including TRPV1, TRPA1, and TRPV4, expressed in sensory neurons, have been implicated in neuropathic pain in rodents. Selective inhibition of TRPV1, TRPA1, or TRPV4 has demonstrated a reduction in PINP, particularly in mechanical allodynia (Basso and Altier, 2017). Beyond the effects on neuronal excitability, neuronal damage appears to play a much significant role in CINP.

Apart from its direct impact on neurons, cisplatin induces a pronounced activation of astrocytes, leading to the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (Shim et al., 2019; Ullah et al., 2021). These cytokines play a role in sensitizing nociceptive neurons and promoting neurogenic inflammation. In addition to astrocyte activation, paclitaxel-induced neuronal damage triggers the infiltration of macrophages into the dorsal root ganglion (DRG) and peripheral nerves (Zhang et al., 2012). Notably, the recruitment and activation of immune cells, coupled with subsequent neurogenic inflammation in the DRG and the spinal cord, occur after the acute phase of paclitaxel treatment. This inflammatory response typically initiates 4–7 days' post-treatment, a period when the initial acute pain has subsided. The delayed onset of inflammation suggests a potential role in the persistence of neuropathic pain symptoms associated with bortezomib-induced neuropathic pain (Uhelski et al., 2021). Vincristine administration leads to the upregulation of matrix metalloproteinase 3 in the DRG (Rajabi and Mousa, 2017), contributing to the demyelination of peripheral nerve fibers. Indicating paclitaxel-induced neuronal damage, the cyclic AMP-dependent transcription factor ATF3, a marker for nerve injury, is robustly expressed in the nuclei of large myelinated DRG neurons post-treatment (Flatters and Bennett, 2006). Additionally, C-C motif chemokine 2 and its receptor, C-C chemokine receptor type 2 (CCR2), are upregulated in sensory neurons during PINP, indicating involvement in

inflammatory processes (Sun et al., 2020). These multifaceted interactions between paclitaxel, immune cells, and neuronal components contribute to the intricate pathophysiology of paclitaxel-induced neuropathic pain.

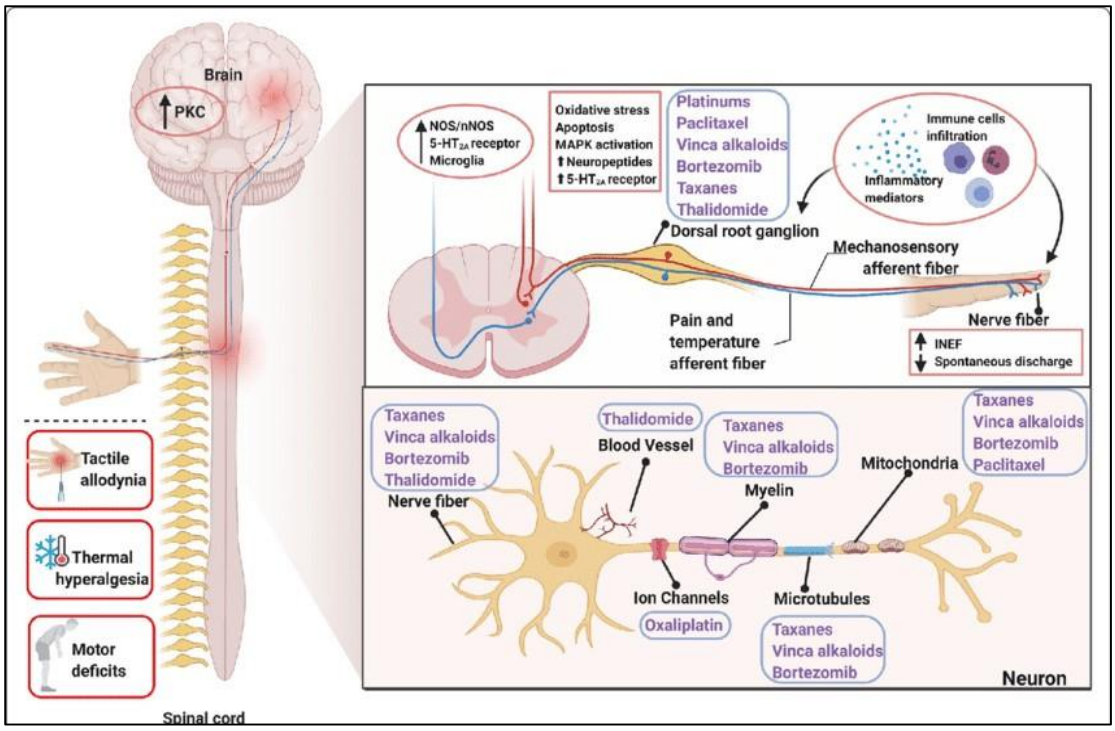

Figure 1.5 Illustrative representation of the mechanism of chemotherapy-induced peripheral neuropathy (CIPN). The sketch-map delineates typical symptoms and targets for CIPN toxicity in the peripheral nervous system, encompassing distal nerve terminals, axonal components (myelin, microtubules, mitochondria, ion channels, and vascular network), the dorsal root ganglion (DRG), and the central nervous system (CNS). CIPN initiation and progression are depicted through chemotherapeutic agents, leading to intraepidermal nerve fiber impairment, abnormal spontaneous discharge, ion channel activation, up-regulation of the neuro-immune system, oxidative stress, and abnormal kinase activation in both the DRG and CNS. The contents within blue boxes represent various chemotherapy agents, and solid dots indicate the targets of the respective chemotherapeutic agents. Reprinted with permission by McGraw Hill LLC from source reference (Yang et al., 2021).

Paclitaxel, originally derived from *Taxus brevifolia* (Pacific yew), serves as a potent antineoplastic agent widely utilized in the therapeutic management of breast, ovarian, and gastric cancers (Boyette-Davis et al., 2015; Höke and Ray, 2014). Notably,

its clinical application is associated with a spectrum of side effects, prominently featuring neuropathy characterized by distal sensory neurotoxicity. A distinctive manifestation of this phenomenon is the emergence of an acute pain syndrome referred to as paclitaxel-associated acute pain syndrome (P-APS), typically reaching its zenith approximately 3-4 days following paclitaxel administration (Baker et al., 2023). One pivotal pathway contributing to paclitaxel-induced neuropathic pain revolves around the perturbation of axonal transport (Figure 1.1). Within sensory neurons, the process of axonal transport crucially hinges upon the dynamic modulation of the cytoskeletal architecture (Baker et al., 2023; Pease-Raissi et al., 2017). Paclitaxel assumes a central role in this context by acting as a tubulin-stabilizing factor. By preventing tubulin depolymerisation, paclitaxel disrupts the delicate equilibrium required for effective axonal transport. The consequence of this interference encompasses a cascade of pathological events, including axonal degradation, axonopathy, and ultimately, loss of epidermal innervation (Figure 1.2) (Höke and Ray, 2014; Kerckhove et al., 2017).

1.3 Pharmacotherapeutics for the treatment of CINP and their limitations

Managing CINP poses a significant challenge for clinicians, with numerous clinical trials and meta-analyses yielding controversial results. Despite decades of dedicated efforts by scientists, there is still a lack of US FDA-approved and therapeutics for the treatment of CINP. The drugs currently employed for neuropathic pain management do not effectively prevent CINP due to the complex and varied molecular and cellular etiologies associated with this condition (Table 1). However, there are several classes of the drugs available which provides only symptomatic relief.

Moreover, for the past few decades' severe side effects are observed with different analgesics which has created a substantial barrier to the pain management.

1.3.1 Tricyclic antidepressants (TCAs)

Despite the proven efficacy of tricyclic antidepressants (TCAs) in managing various forms of painful neuropathy and polyneuropathy, limited clinical trials have demonstrated positive effects in alleviating CINP. Nortriptyline, specifically, did not exhibit effectiveness in reducing pain associated with cisplatin-induced CINP. Furthermore, the leading compound in this class, amitriptyline, showed no significant difference from a placebo in ameliorating neuropathic pain induced by taxane, platinum, or vinca alkaloids in double-blind randomized controlled trials (RCTs) (Jain et al., 2023).

1.3.2 Serotonin–noradrenaline reuptake inhibitors (SNRIs)

Considering the involvement of both serotonergic and noradrenergic signaling in chemotherapy-induced neuropathic pain (CINP), serotonin–noradrenaline reuptake inhibitors (SNRIs) present as promising candidates for treatment. Notably, venlafaxine and duloxetine have shown positive outcomes in mitigating CINP. In the realm of preclinical studies, the administration of venlafaxine has demonstrated a reduction in CINP in rats (Sachau et al., 2021). Moreover, venlafaxine exhibited efficacy in diminishing oxaliplatin-induced CINP in a small randomized controlled trial (RCT). A recent phase III RCT involving 231 participants observed significant improvements in CINP with duloxetine. Stratifying patient groups based on the cytostatic substance received, revealed duloxetine's heightened efficacy in alleviating oxaliplatin-induced CINP compared to taxane-induced CINP. Duloxetine, without impacting glial

activation and cytokine release in the spinal cord, demonstrates the ability to maintain neurotransmitter balance, thereby alleviating the pathophysiological neuronal effects of oxaliplatin (Ozols, 2000). The prominence of neuronal damage in oxaliplatin-induced CINP, in contrast to the robust inflammatory component in taxane-induced CINP involving the dorsal root ganglion (DRG) and spinal cord, contributes to the varying efficacies of duloxetine and amitriptyline. These differences may stem from distinct affinities for noradrenaline transporters, resulting in divergent concentrations of synaptic noradrenaline. Additionally, the effects of amitriptyline on muscarinic, histaminergic, and adrenergic receptors further contribute to the divergent outcomes in reducing CINP. This nuanced understanding of the pharmacological and mechanistic disparities between duloxetine and amitriptyline enhances our grasp of their respective roles in addressing the intricate landscape of CINP.

1.3.3 Anticonvulsants

Anticonvulsants play a crucial role in managing various forms of chronic pain, particularly those stemming from CINP. Anticonvulsants such as carbamazepine, oxcarbazepine, topiramate, gabapentin, pregabalin, and lamotrigine are commonly employed for pain management. Specifically, for CINP, gabapentin and pregabalin are frequently prescribed due to their demonstrated effectiveness in alleviating pain associated with chemotherapy. These drugs not only modulate intracellular calcium levels but also exhibit additional activity on substance P and calcitonin gene-related peptide (CGRP), which are critical elements in the neuropathic pain signaling pathway induced by chemotherapy agents (Zengin et al., 2019). Despite their therapeutic benefits, anticonvulsants may elicit common side effects such as sedation, dizziness, memory loss, edema, and dry mouth. The occurrence of these side effects prompts

careful consideration by clinicians and scientists regarding the extent of anticonvulsant use in managing pain induced by chemotherapy.

1.3.4 Opioids and NSAIDs

Several trials have indicated positive outcomes with tramadol or controlled-release oxycodone in patients experiencing neuropathic pain (Blanton et al., 2019). Tapentadol, characterized as a selective μ opioid receptor agonist and noradrenaline reuptake inhibitor, holds promise for potentially greater efficacy in alleviating CINP compared to traditional opioids. The investigation of buprenorphine in CINP is also warranted due to its ability to block μ opioid and κ opioid receptors, NMDARs, and enhance voltage-gated potassium channel activity in neurons. On the other hand, nonsteroidal anti-inflammatory drugs (NSAIDs) are not recommended for CINP as they do not effectively target neuronal hyper-excitability, a crucial pathophysiological mechanism in CINP but few NSAIDs may provide for symptomatic relief (Stoicea et al., 2015).

1.3.5 Anti-inflammatory compounds

The prevention of neurogenic inflammation and subsequent persistent pain through the inhibition of glial cell activation has been proposed as a potential strategy for mitigating CINP. Non-steroidal anti-inflammatory drugs (NSAIDs) may exert a neuroprotective effect through the inhibition of cyclooxygenase-2 (COX-2), a key player in neuropathic pain. In the context of neuropathic pain, COX-2 up-regulation contributes to the increased expression of ion channels and cytokines. In a rat model for CINP, studies have demonstrated the up-regulation of COX-2 in the dorsal horn post 7 days' chemotherapy administration. This suggests that the neuroprotective

effects of NSAIDs, achieved through COX-2 inhibition, could potentially attenuate the molecular mechanisms associated with neuropathic pain, offering a therapeutic avenue for managing CINP (Micallef et al., 2020). Combining duloxetine with an anti-inflammatory agent is theoretically appealing to improve CINP induced by paclitaxel or vincristine (Leelaprakash and Mohan Dass, 2011). Incorporating these anti-inflammatory compounds into the therapeutic armamentarium for CINP not only provides avenues for pain relief but also underscores the importance of a multi-faceted approach in addressing the diverse mechanisms underlying CINP. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and diclofenac, work by inhibiting enzymes involved in the production of inflammatory mediators. By reducing prostaglandin synthesis, NSAIDs contribute to a diminished pain response during chemotherapy. Corticosteroids, exemplified by dexamethasone, showcase anti-inflammatory properties that extend beyond their conventional use. These compounds modulate immune responses, effectively dampening neuroinflammation associated with CINP and providing relief from pain. TNF-α inhibitors, including infliximab and etanercept, emerge as promising candidates for CINP management by specifically targeting the inflammatory cytokines involved in the pain signaling cascade. Omega-3 fatty acids found in fish oil, recognized for their anti-inflammatory properties, hold promise in modulating inflammatory pathways and contributing to the amelioration of CINP. Minocycline, an antibiotic with anti-inflammatory effects, exhibits promise in preclinical studies for attenuating CINP. Its ability to inhibit microglial activation and reduce pro-inflammatory cytokines positions it as a potential adjunct in CINP management. A more promising avenue involves inhibiting the p38–MAPK pathway, known to activate microglia, astrocytes, and satellite glial cells, causing sensitization of

nociceptive neurons an attractive dual target for neuropathic pain treatment. The role of TNF in the pathogenesis of chemotherapeutic agents suggests that inhibiting TNF-α signaling may reduce the inflammatory component of CINP. Blockade of TNF signaling, exemplified by infliximab a TNF-α blocking chimeric monoclonal antibody has shown benefit in patients with neurosarcoidosis, a neuropathic pain syndrome with a pronounced inflammatory contribution due to granuloma formation. Infliximab, as an adjunct to duloxetine, may hold promise in improving CINP induced by paclitaxel or vincristine (Lian et al., 2021).

1.4 Perspectives from preclinical data

The development of new therapeutics for CINP involves two primary strategies: compounds targeting inflammatory processes and those modulating the excitability of peripheral sensory neurons by inhibiting voltage-gated or ligand-gated ion channels. Additionally, there is a focus on neurotransmitter reuptake inhibitors, as well as opioid and cannabinoid receptor agonists.

1.4.1 TRP channel inhibition

Considering the significant role of TRP channels in CINP, antagonists targeting TRP channels present a potential avenue for inhibiting the transmission of neuropathic pain from the periphery to the spinal cord. Second-generation TRPV1 antagonists are currently undergoing clinical trial and may become available in the near future. Endogenous lipidergic TRPV1 inhibitors, such as neuroprotectin D1 and resolvin E1, have demonstrated efficacy in reducing microglial and astrocytic activation, subsequently inhibiting cytokine release following nerve injury in murine models (Meesawatsom et al., 2020). The synergistic mechanism of TRP channel inhibition

coupled with the suppression of glial cell activation holds potential for CINP treatment, particularly in paclitaxel, vincristine and cisplatin-induced neuropathic pain, where robust gliosis and increased TRPV1/TRPA1/TRPM8 activation are implicated.

1.4.2 TRPA1 and TRPV4 modulation

In addition to TRPV1, the roles of TRPA1 and TRPV4 channels in CINP have garnered attention. These channels contribute to the transduction of noxious stimuli and may serve as potential targets for therapeutic intervention. Modulation of TRPA1 and TRPV4 channels could offer a complementary approach to address the diverse mechanisms underlying CINP. The hyperpolarization-activated cyclic nucleotide-gated channels HCN1 and HCN2 have recently been shown to be involved in neuropathic pain. Because HCN2 is also markedly upregulated during oxaliplatin CINP, HCN2 antagonists might represent promising substances specifically for the treatment of oxaliplatin CINP. GSK2798745, a novel TRPV4 antagonist, has demonstrated favorable tolerability in Phase I clinical trials involving healthy volunteers. Administration of this compound did not elicit adverse effects, suggesting the potential of TRPV4 antagonists as a therapeutic approach for various pain conditions, including inflammatory pain, neuropathic pain, and CINP (Liedtke and Kim, 2005). In Phase I trials, TRPV4 knockout (Trpv4−/−) mice displayed normal heat and touch sensation. However, in CINP models, these mice exhibited reduced mechanical allodynia, hyposmotic solution-induced nociception, diminished edema formation, and decreased cytokine release. A fly model of neuropathy further revealed disruptions in axonal interactions and dendritic degeneration due to mutations within the TRPV4 gene. The paclitaxel-induced peripheral neuropathy model was initially employed to investigate TRPV4 involvement in nociceptive responses to mechanical and hypotonic stimulation of the hind paw (Marwaha et al., 2016a). Additionally, the injection of the integrin

antagonist peptide GRGDTP into the hind paw resulted in a reduction of both mechanical hyperalgesia and hypotonicity-induced nociception in groups subjected to paclitaxel injection. In a subsequent study by the same author, an exploration of the role of TRPV4 in mechanical hyperalgesia and hypotonicity-induced nociceptive behavior induced by both paclitaxel and vincristine was conducted. These findings contribute to a more comprehensive understanding of the intricate molecular mechanisms involved in neuropathic pain associated with chemotherapy. Additionally, injection of the integrin antagonist peptide GRGDTP in the hind paw reduced mechanical hyperalgesia and hypotonicity-induced nociception in paclitaxel-injected groups. In a subsequent study, the same author explored TRPV4's role in mechanical hyperalgesia and hypotonicity-induced nociceptive behavior induced by paclitaxel and vincristine. Intrathecal treatment with TRPV4 as oligodeoxynucleotide attenuated vincristine-induced mechanical allodynia and hypotonicity-induced nociception. Moreover, only Trpv4+/+ mice exhibited mechanical hyperalgesia following chemotherapy administration, while Trpv4−/− mice were protected from the induction of nociception in both paclitaxel and vincristine models of CINP (Ding et al., 2010; Todaka et al., 2004). However, further research is needed to elucidate the specific involvement of TRPA1 and TRPV4 in CINP, and the development of selective modulators for these channels could present new opportunities for therapeutic advancements in the field.

1.4.3 Antioxidants and neuroprotective agents

The implication of reactive oxygen species (ROS) and their oxidation products in CINP induced by various chemotherapeutic substances raises the hypothesis that early intervention with antioxidants could mitigate the neurotoxic effects of ROS and alleviate CINP. Despite the promise of this approach, the use of antioxidants is intricate due to their distinct sites of action within cells and tissues, as well as specific

distribution routes in the body, influenced by factors such as lipophilicity and solubility. One example is Phenyl-N-tert-butyl-α-phenylnitrone, a free radical scavenger, which demonstrated efficacy in reducing paclitaxel-induced mechanical allodynia in a rodent study (Zaki et al., 2022). The endogenous lipophilic antioxidant α-tocopherol (vitamin E) was initially considered to ameliorate platinum-induced CINP, and showed promising effects at doses of 300–600 mg/day, mostly in randomized trials one of which was also placebo-controlled with small number of patients. The neuroprotective, anti-inflammatory and anti-nociceptive effects of omega 3 fatty acids derived from docosahexaenoid acid and eicosapentaenoic acid have been demonstrated in many pain-relevant contexts. A small double-blind RCT involving 57 participants investigated whether pearls containing omega 3 lipids (640 mg three times per day) could ameliorate paclitaxel-induced CINP. Compared with placebo, the omega-3 lipid treatment was associated with a significant reduction in the incidence of paclitaxel CINP.

1.4.4 NMDA antagonists

NMDA antagonists such as ketamine, amantadine, and dextromethorphan are emerging as novel therapeutics for pain management (Bunch and Qian, 2017). These drugs act on central sensitization and prevent neuronal excitotoxicity via inhibiting the NMDARs at spinal and supraspinal levels. Despite their proven preclinical efficacy, the NMDARs fails to treat pain condition in clinics adequately and produce several CNS toxicities. The adverse effects mainly got precipitated as antagonizing the NMDARs leads to the modifications in basal physiological role of these receptor systems and cross interactions between intracellular signaling.

1.5 Other pharmacological treatment

Other strategies available for the management of CINP are low dose naltrexone, topical agents (diclofenac, lidocaine, and capsaicin), skeletal muscle relaxants (baclofen, tizanidine, cyclobenzaprine, on botulinum toxin A, cannabinoids, etc. which still require further studies to establish a better therapeutic approach such as combination therapy (Bunch and Qian, 2017).

In light of the abundant evidence garnered from both clinical and preclinical studies, there is an urgent need to identify novel and reliable molecular targets for the treatment of CINP. Currently, the intricate mechanisms by which specific chemotherapy drugs induce pain and neuropathy remain elusive, significantly hindering the development of efficacious anti-pain therapies. TRP channels, exhibiting promise as effective molecular targets, warrant careful exploration.

Drugs	Anti-cholinergic	Addiction	Sedation, Drowsiness	Nausea, GI upset or bleeding	Insomnia, Agitation	Weight gain	Orthostatic hypotension
SNRIs							
Tricyclic Antidepressants							
Anticonvulsants							
Topical Products							
NSAIDs							
Opioids							

Table: 1 Currently available drugs for the treatment of CINP and their limitations [*]***Darker red color represents increased risk and severity of the side effect.***

1.6 TRP channels as a potential drug target for CINP

Pain results from complex processing of neural signals at different levels and TRP channels are molecular sensors for noxious mechanical, chemical, and thermal insults. Activation of peripheral nociceptors by TRP channels, especially the capsaicin receptor TRPV1 and wasabi receptor TRPA1, initiates neurogenic inflammation and pain sensation. The mammalian TRP family members are classified into six subfamilies based on the sequence of amino acid homology (Dai, 2016; Marwaha et al., 2016b) namely TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin). Their structure possesses six fringe extracellular domains with N and C-terminals and intracellular regions with a pore loop between the 5th and 6th transmembrane domains (Buijs and McNaughton, 2020; McKemy, 2013; Nilius and Voets, 2005; Sadowska et al., 2019; Takayama et al., 2019). Four sub-units as homo and/or heterotetramers are responsible for forming a functional loop. Far from their distinguished membrane topography and the penetration rate of cations, the TRP channels are strikingly complex in contrast with other ion channel families. The concurrent homology of mammalian TRPs is small and has a wide variety of triggering stimuli such as temperature, chemical, osmolarity, mechanical stimulus, lipids, light, oxidative burst, acid, and pheromones (Ramsey et al., 2006). These cationic channels also include a sensory transducer which causes sensitization and development of chronic pain induced by thermal, mechanical, and chemical stimuli. At the cellular and molecular level, TRPV1/2/3 and TRPM8 behave as thermoreceptors and TRPV4 and TRPA1 are referred to as mechanoreceptors (Akopian, 2016; Hung and Tan, 2018; Liedtke and Kim, 2005). Furthermore, polymodality also exists such as TRPV1, TRPV3, TRPA1, and TRPM8 which are also noted as chemoreceptors (Figure

1.6). The TRPs channels are essential molecular players in acute- inflammation and chronic pain states.

1.6.1 Growing significance of TRPV1 channels in CINP

Transient receptor vanilloid 1 receptor (TRPV1) is expressed in nearly 60% of peptidergic small-diameter primary nociceptors in the dorsal root ganglia (DRG) and trigeminal ganglia, which sense environmental cues in the skin and many visceral organs (Andrade et al., 2012). TRPV1 is a nonselective cation channel that is activated by capsaicin, noxious heat, acid and many endogenous ligands as well as plant-derived natural compounds. In addition to direct activation, TRPV1 is also sensitized by activation of GPCRs and tyrosine kinase receptors through intracellular signal transduction pathways involving activation of many protein kinases, including protein kinase C (PKC), protein kinase A (PKA), and phosphoinositide 3-kinase (PI3K) (Hong et al., 2019). Both activation and sensitization of TRPV1 lead to enhanced pain responses. Both genetic ablation and pharmacological inhibition studies have provided convincing evidence that TRPV1 significantly contributes to both chronic inflammatory pain and neuropathic pain resulting from chemotherapy (Patil et al., 2020). Moreover, both TRPV1 and TRPA1 have been shown to be involved in the pathogenesis of CIPN. TRPV1 act as contributors to the induction of CINP and spinal astrocytes and microglia are also involved in the initiation and maintenance of oxaliplatin-induced CINP (Chukyo et al., 2018). A report on neuropathic pain triggered by paclitaxel (PAC) was documented. In their study, the authors explored the downstream signaling of TRPV1 and used multiple approaches, such as antagonist and agonist and TRPV1-KO mice (Luo et al., 2019b). The study suggested that the paclitaxel significantly increases the activation and expression of the TRPV1 mRNA

level, further downstream enzymes such as PLC, PKC5, and PKA get over-activated which can cause allodynia and hyperalgesia. This was established when rat spinal cord slices were processed for in-vitro incubation with PAC (100 nM) and TRPV1 antagonists (SB366791 and AMG9810) (Hong et al., 2017; Kamata et al., 2020; Thakre and Bellingham, 2017; S. Wang et al., 2018). The PAC resulted in upregulation of c-Fos nuclear expression in dorsal horn neurons that was reduced by SB366791 and AMG9810 which further explains the role of TRPV1 as a key player in the CINP. A study was suggested that vincristine-treated rats developed mechanical allodynia/hyperalgesia and increased levels of TRPV1 protein expression in the DRG. They speculate that vincristine-induced neuropathic pain may be the result of up-regulation of TRPV1 protein expression in DRG neurons. Moreover, DRG sections were double-labeled for TRPV1 and the small-diameter DRG neuron marker, isolectin B4. The ratio of neurons expressing TRPV1 among those that were isolectin B4-positive was significantly higher in vincristine-treated rats, confirming that a considerable number of C-fiber neurons began to express TRPV1 after vincristine treatment (Hermes et al., 2016). Breese and his colleagues showed that isolectin B4-positive small-diameter DRG neurons show increased TRPV1 function and expression after peripheral inflammation. Both studies confirmed that vincristine may have increased the expression of TRPV1 in small-diameter DRG neurons, and the up-regulation of TRPV1 due to the onset of vincristine-induced mechanical allodynia/hyperalgesia (Khan et al., 2021). Therefore, TRPV1 likely plays an important role in vincristine-induced peripheral neuropathic pain.

1.6.2 Crucial role of TRPA1 channels as regulators in CINP

TRP family cationic channels are widely expressed across the PNS and CNS, and plays major role in the pain modulation. Several lines of evidence suggest that TRPA1 and TRPV1 mutually control the pain signals transduction of noxious stimuli in chemotherapy sensitized sensory neurons (Adamek et al., 2019). A study revealed that both TRPV1 and TRPA1 nociceptors are co-expressed majorly in the DRG and spinal tissues however they are also located in other cells including keratinocytes, microglia and dendritic cells (Chukyo et al., 2018). Positive interaction between TRPA1 and TRPV1 is dependent on extracellular Ca2+ ions, indicating that activation of certain intracellular secondary signalling pathways coupled with $Ca2^{+}$ influx regulates the activity of these nociceptive channels. Therefore, it seems plausible that the $Ca2^{+}$ influx through the opening of TRPV1 may in turn potentiate the sensitivity of TRPA1 located in the close vicinity (Chen et al., 2011). Allyl isothiocyanate (mustard oil or AITC) induced thermal hyperalgesia in rodents and humans involved the sensitization of TRPV1 which is dependent on TRPA1 mediated mechanism (Jordt et al., 2004). Moreover, the whole cell patch-clamp recording and intracellular calcium imaging data using HEK293T cells over expressing mouse TRPV1/TRPA1 indicated the increased activation of both the channels post thermal stimulus application in presence of AITC as compared to the control condition. Calcium imaging of isolated sensory neurons from TRPA1 KO mice suggests that AITC increases the thermal response specifically in the subpopulation expressing TRPV1. Furthermore, this effect was abolished by TRPV1 inhibitors capsazepine and effectively absent in sensory neurons from TRPA1 KO mice (Vandewauw et al., 2018). The data suggests that TRPV1 sensitization was mediated through TRPA1 dependent pathways in the sensory

neurons. Oxaliplatin treatment increases the co-expression of both TRPV1 and TRPA1 receptors in small-sized DRG and unmyelinated C-fibres neurons which is coupled with acute cold hyperalgesia (Chukyo et al., 2018). Another study suggested that administration of oxaliplatin in the DRTGB cell line causes an elevation the levels of free radical generation (ROS & RNS) as well as an increase the expression of both TRPV1 and TRPA1 in DRGs and spinal tissues. Furthermore, the TRPA1 antagonist (A-967079) also inhibit the expression of TRPV1 in chemotherapy sensitized sensory neurons. In addition, in-situ hybridization analysis revealed that co-localization of both receptors in the neuronal cells indicated that both receptors co-expressed in the same neuronal cells (Chen et al., 2011). Paclitaxel treatment also leads the thermal hyperalgesia by up-regulating co-expression of the both nociceptive receptors in the DRGs and spinal tissue levels. Furthermore, TRPV1 inhibitor capsazepine suppressed the TRPV1 and TRPA1 protein level in the DRGs and spinal level (Sałat and Filipek, 2015; Srebro et al., 2016).

1.6.3 Unveiling the significance of TRPM8 channel in CINP

TRPM8, belonging to the transient receptor potential (TRP) family, is expressed in primary sensory neurons within the DRG. In the realm of neurobiological investigations, TRPM8 emerges as a pivotal factor influencing hypersensitivity to both thermal and mechanical stimuli. This influence is particularly notable in conditions involving inflammation and peripheral nerve injury induced by specific chemotherapeutic agents such as oxaliplatin, paclitaxel, and vincristine. Functioning as a cold-sensitive ion channel, TRPM8, in tandem with TRPA1, is recognized as a cold transducer (Shibata and Tang, 2021). Notably, the administration of oxaliplatin has been linked to the emergence of acute cold hypersensitivity in rats. The expression of

TRPM8 mRNA in DRG neurons is specifically implicated in the onset of oxaliplatin-induced acute cold hyperalgesia. Experiments involving TRPM8-deficient mice underscore the crucial role of TRPM8 in cold hypersensitivity, as these mice exhibit diminished behavioral responses to cold stimulation. Additionally, both *in vitro* and *in vivo* studies reveal an elevation in the levels of TRPM8 mRNA in DRG cells following oxaliplatin treatment (Basso and Altier, 2017). Collectively, these insights highlight the significance of TRPM8 in the early stages of oxaliplatin-induced peripheral neuropathy, providing valuable contributions to the comprehension of the molecular mechanisms that underlie chemotherapy-induced neuropathic pain.

1.6.4 TRP channels and cytokines/chemokines interplay in CINP

CINP involves neuro-inflammatory reactions. The number of inflammatory cells that accumulate across damaged nerves in response to the stimulation of Schwann cell and resident macrophages, produces numerous cytokines and chemokines. These include TNF-α, IL-1β, IL-6, IL-8, and CCL2, etc (Brandolini et al., 2019; Zhou et al., 2016). Antineoplastic agents such as paclitaxel, cisplatin, and vincristine cause an increase in the synthesis and secretion of pro-inflammatory cytokines such as TNF-α and IL-1β and chemokines such as MCP-1 through TRPV1 dependent manner (Vom Braucke et al., 2020). These inflammatory cytokines may bind to neuronal and glial cell receptors to increase pain-like activity, thus resulting in a possible self-sustaining cycle. TRPV1 plays a key role in the inflammatory sensitization of afferent sensory and somatosensory nerves fibers. There are many pro-inflammatory cytokines including TNF-α and IL-1β that activate signal transmission pathways in sensory neurons leading to downstream activation/sensitization of the TRPV1 channel (Dhukhwa et al., 2019). Thalidomide is an inhibitor of the TNF-α synthesis, ameliorates vincristine-induced

TRPV1 expression at a translational level (Y. Wang et al., 2018). TNF-α can decrease GABAergic inhibitory signaling in the spinal cord via a MAPK (p38)-mediated pathway to enhance pain signaling (Tang et al., 2021). TNFα is synthesized and released by many cells, however, in the context of CINP, it was documented that the elevation of TNF-α occurs predominantly in macrophages. A study suggested that peripheral sensitization is marked by increased TRPV1 activity in response to TNF-α and these increased ion channel responses result from p38-MAPK activation in DRG neurons (D. Zhang et al., 2020). Changes in the TNF-α expression have been observed in specific animal models of CINP both peripherally and centrally (Xu et al., 2016; Zhang et al., 2016). A growing amount of evidence suggests that the interplay of TRPV1 and cytokines play a crucial role in the CINP. TRPV1 was the downstream target on which the increased expression of IL-6 in DRG neurons exerted its effects associated with the development of CINP. A study has shown the involvement of IL-6/JAK/PI3K/TRPV1 signaling cascade in the development of peripheral sensitization in CINP (Fang et al., 2015). TNF-α has a crucial role in the formation and sensitization of peripheral afferents by triggering neuronal TNF-α receptors to increase the production of IL-1β, IL-6, and CCL2 [generally known as monocytes chemoattractant protein-1 (MCP-1)]. Several preclinical findings demonstrated the role of TRP channels including TRPA1, TRPV1 and TRPM8 as major contributors to thermal, mechanical and cold hypersensitivity during chemotherapy-induced neuropathic pain. TRPA1 activation and sensitization is accompanied by increased expression of TRPV1, calcitonin gene-related peptide (CGRP), Substance-P and release of pro-inflammatory cytokines in the DRG and spinal cord tissues (Schecterson et al., 2020; Takayama et al., 2019). Another study demonstrated the upregulation of TRPA1, TRPV1 and

TRPM8 co-expression in oxaliplatin-induced neuropathic pain conditions and inhibition of both polymodal nociceptors attenuates pain-like behavior in CINP animals (Chukyo et al., 2018). Treatment with paclitaxel, bortezomib and cisplatin led to an increase in expression of both TRPA1/TRPV1 mediated mitogen-activated protein kinases (MAPK) pathways followed by the release of pro-inflammatory cytokines and chemokines resulting in neurogenic inflammation (Ikeda-Miyagawa et al., 2015). The oxaliplatin (OXL) shows a dose-related reduction of the density and length of neurites and neuronal number. These cellular changes have led to enhanced cAMP levels due to TRPV1/TRPA1 sensitization after acute or chronic treatment with oxaliplatin (Anand et al., 2010).

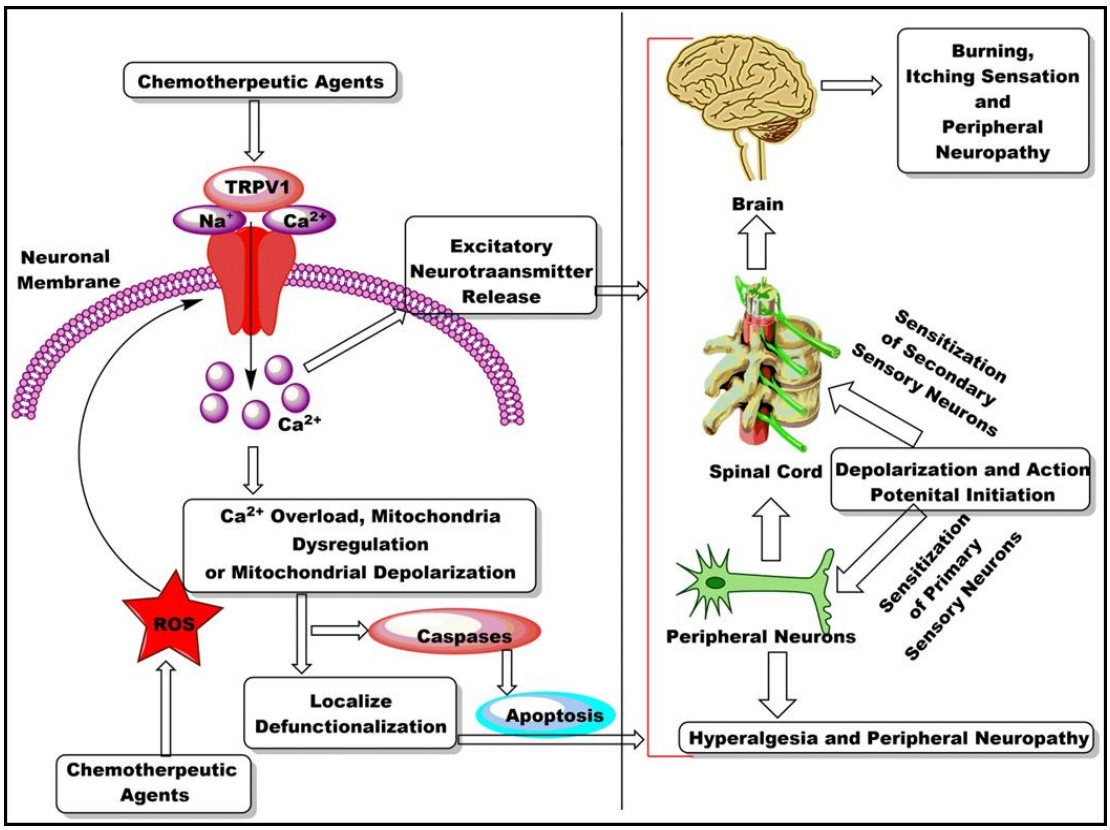

Figure 1.6 A Mechanistic understanding of the TRP channels to induce CINP: Simplified model of chemotherapeutic agents may impair mitochondrial functions both directly and indirectly by evoking Ca^{2+} influx through TRPV1/TRPA1 and

further causes the peripheral and central sensitization in the CINP. Reprinted with permission from Life Sciences from own reference sources (Akhilesh et al., 2022).

1.7 NMDA receptors system: A key player in the pathophysiology of CINP

NMDA receptors are ionotropic, excitatory, heteromeric glutamate receptors. In the central nervous system (CNS) of mammals, fast synaptic transmission is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainite receptors while the slow synaptic transmission is mediated by NMDA receptors. NMDA receptors have been widely implicated in the transmission of both nociceptive and neuropathic pain (Li et al., 2019). The NMDA receptors are abundantly distributed in the pain pathway and are responsible for synaptic plasticity corresponding to chronic pain (Laumet et al., 2017). Painful stimuli cause the activation of afferent myelinated Aδ and unmyelinated C fibers that cause a release of glutamate along with substance P and neurokinin A in the dorsal horn of the spinal cord. This makes the postsynaptic neurons fire that project signals to the supraspinal structures of the CNS. The thalamus acts as a relay station and distributes the afferent signals to different cortical regions specifically the anterior cingulate cortex (ACC) and the insular cortex (IC) that are responsible for the unpleasant feeling of pain. Hence, as a consequence of these series of events the endogenous pain modulation mechanisms are activated.

1.7.1 NMDA mediated central sensitization across the ascending and descending pain pathways

Central sensitization is a key term used to demonstrate the pathophysiology of chronic pain. The neuronal transmission during central sensitization gets rapidly increased among the excitatory neurons while the activity of inhibitory neurons gets

decreased. Moreover, the regulation of pain via endogenous mechanisms of a body system is impaired throughout the descending pain pathway. Various ion channels (e.g., TRPV1, TRPA1, Nav1.6, etc.), secondary messenger pathways (e.g., PI3K/Akt, CREB, mTOR, ERK 1/2), cytokines (e.g., IL6, IL1β, TNFα, NFKβ, etc.) and other mediators (VEGF, oxidative stress, histamine, serotonin, etc.) are involved in the development and maintenance of central sensitization (Ji et al., 2018). One of the key receptor systems that closely regulates this central neuron-based phenomenon is NMDA (Figure 1.7). The NMDA receptor-mediated long-term potentiation (LTP) has been observed in the pain pathways across the neurons residing in the spinal dorsal horn (Li et al., 2019). N-methyl-D-aspartate receptors (NMDARs) are excitatory receptors, and a subset of this receptor unit, NR2B, is essential for NMDAR localization within the synaptic membrane and plays a vital role in learning, memory, and nociceptive pain (Zhou et al., 2011). Chemotherapy drugs such as paclitaxel, vincristine, and oxaliplatin are known to cause neuropathic pain by activating presynaptic NMDA receptors (NMDARs) in the spinal cord, which amplifies pain signals. This activation is connected to ion channels called TRP channels, specifically TRPV1 (Xie et al., 2017; Zafari et al., 2023). These TRPV1 channels interact with NMDARs and play a crucial role in their function. Paclitaxel, in particular, intensifies the interaction between TRPV1 and NMDARs, leading to changes in spinal cord synapses that contribute to painful neuropathy. This process triggers the activation of PKC (protein kinase C), which further stimulates NMDARs, worsening the neuropathic pain. Reactive oxygen species (ROS) enhance the spontaneous release of glutamate from presynaptic terminals through activation of TRPA1 and TRPV1 channels (Huang et al., 2019; Lin et al., 2019). Overactivity of these ion channels due to ROS may lead to central

sensitization through NR2B in the spinal cord, resulting in chronic pain following paclitaxel administration. Central sensitization is a key feature for the pathogenesis of peripheral neuropathy induced by NMDAR hyperactivation at the spinal and supraspinal levels. NMDAR is known to transmit potent nociceptive signals by interacting with TRPA1, TRRPV1, and TRPM8 nociceptors (Huang et al., 2019; Sadler and Stucky, 2019).

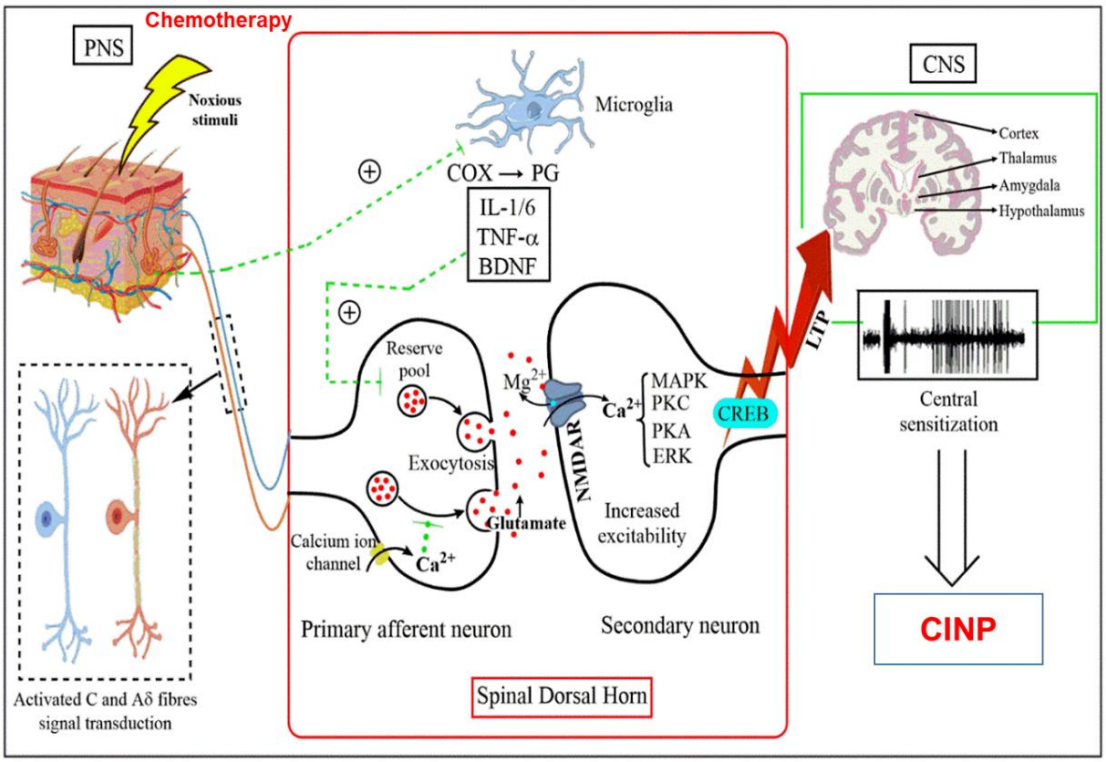

Figure 1.7 Role of NMDA receptor system in the neurobiology of CINP. Intense or persistent injury by noxious stimuli causes depolarization of C and Aδ fibers. Excitation of these fibers leads to exocytosis of a reserve pool of excitatory neurotransmitter, glutamate, into the synaptic cleft. The excess release of glutamate in the synaptic cleft increases the activity of NMDAR in the secondary neurons. Glutamate binds to the glutamate binding site of NMDAR, releasing the magnesium ion block and allowing calcium ion influx into the cell. Hence, raising the intracellular calcium ion concentration in the neuron. A high concentration of calcium ions results in the activation of different secondary messengers such MAPK, PKA, PKC, and ERK, activating different cascades of calcium-dependent signaling pathways. This series of events facilitates the transmission of pain signals to the brain by increasing the excitability of output neurons. The prolonged damage to peripheral nerves activates the

resident macrophage cells, microglia, releasing a host of cytokines and producing prostaglandins, further contributing to central excitation. Reprinted with permission by American Chemical Society from source reference (Uniyal et al., 2021b).

The potentiated function of neurons in the central nociceptive pathways is attributed to the membrane hyperexcitability, highly efficient synaptic transmission, reduced inhibition resulting from profound plasticity in the somatosensory nervous system. This state characteristically portrays central sensitization that arises from nerve injury or inflammation (Latremoliere and Woolf, 2009). The most investigated form of the aforementioned profound plasticity is the NMDA-mediated LTP, evident in higher CNS regions and the central synapses responsible for perception and transmission of sensory signals (Li et al., 2019). Investigating the spinal dorsal horn mechanism that accounts for LTP has been difficult due to the sheer complexity of the spinal neural network and the difficulty in accurate slicing. However, whole-cell and intracellular patch-clamp studies have revealed that both increased synaptic activity and increased postsynaptic depolarization lead to LTP (Ikeda et al., 2003). Development of LTP in the spinal dorsal horn requires activation of the NMDA receptor that results in high Ca^{2+} influx. While the release of substance P and calcitonin gene-related peptide (CGRP) after nerve injury is believed to potentiate the current modulated by NMDA receptors in the neurons. Substance P binds to the neurokinin-1 receptor to induce prolonged membrane depolarization. This prolonged membrane depolarization removes the Mg^{2+} block from the NMDA receptors, causing an influx of Ca^{2+} ions (Li et al., 2019). CGRP in particular potentiates the activity of substance P and activates protein kinase A (PKA) and protein kinase C (PKC) by binding to postsynaptic CGRP1 receptors (Sun et al., 2004). CGRP also causes the enhanced release of brain-derived neurotrophic factor (BDNF) that binds to its high-affinity tropomyosin receptor kinase

B (trkB) receptors that result in the activation of extracellular signal-regulated kinase (ERK) and PKC pathways and enhancing C-fiber responses mediated by NMDA receptor (Zhou et al., 2008). Activation of PKC, PKA, ERK, and calcium/calmodulin-dependent protein kinase II (CaMKII) causes a.) phosphorylation that decreases the threshold and activation kinetics of NMDA and AMPA receptors resulting in increased signal transmission; b.) ERK phosphorylation decreasing K^+ currents leading to enhanced neuronal excitability; c.) ERK, PKC, and CaMKII cause the trafficking of NR1-containing AMPA to the membrane; and d.) Activation of cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) pathway to enhance gene expression that results in strengthened synapses (Latremoliere and Woolf, 2009). PKA or PKC phosphorylation of NR1 results in an increased response of NMDA receptors to glutamate, thereby causing hyperexcitability leading to the central sensitization and chronic pain. Moreover, COX and prostaglandin pathways are involved in the peripheral as well as central sensitization (McCrory and Fitzgerald, 2004). Bradykinin, an inflammatory peptide was observed to activate NMDA receptor activity in the spinal dorsal horn and by thus promoting hypersensitivity (Kohno et al., 2008). The mechanism behind this phenomenon was the activation of bradykinin B2 receptors followed by the activation of phospholipase C_β and phospholipase A_2 (PLA_2). The PLA_2 stimulates the arachidonic acid which further gets converted to the PGE2 by the action of COX and prostaglandins (Kohno et al., 2008). Finally, the PGE2 activates PKA and PKC that phosphorylates the NMDAR, regulates NMDAR trafficking to the membrane, and alters their kinetic properties.

1.7.2 NR2B assembly subunit of NMDA critically mediates CINP

NR2B subunit is very essential for making the NMDA receptors functional after getting assembled into a working system. This subunit is highly distinct from other subunits of NMDARs as it consists of a larger intracellular c-terminal tail. The NR2B subunit of the NMDA receptor system is highly expressed across the descending and ascending pain pathways and several animal models of chronic pain have demonstrated a strong reproducibility to this pipeline. A recent study has reported that the NR2B subunit phosphorylation regulates synaptic plasticity under migraine condition (Wang et al., 2020). Furthermore, the animal model of peripheral nerve injury, cancer pain, and chemotherapy-induced neuropathic pain have also suggested the critical involvement of this subunit in the progression of chronic pain (Chia et al., 2020a; Wu et al., 2018; Yang et al., 2018). Moreover, peripheral nerve injury decreases the expression of GluN1 (NR1) and increases the expression of GluN2B (NR2B) in the DRG (Laumet et al., 2015). During chronic pain condition the hyperactivity of presynaptic NMDA receptors at primary afferent neurons results in the elevated release of glutamate (excitatory neurotransmitter) into the spinal dorsal horn (Gill et al., 2015). In the DRG, presynaptic NMDA receptors are more resilient to Mg^{2+} block and suppression of action potential as compared to the postsynaptic NMDA receptors (Gill et al., 2015). The NR2B causes the depolarization and excitatory post-synaptic potential after activation due to the binding of the pre-synaptic glutamate. When the threshold is attained due to temporal and spatial summation the Mg^{2+} get dissociated from NR2B. Postsynaptic elevation of Ca^{2+} influx causes calcium-calmodulin binding that initiates several downstream pathways. This ultimately results in the sustained excitatory post-synaptic potential which causes LTP via 1) CamKII>PKA>cAMP>CREB and 2)

MAPK pathways (Mohd Noh and Ismail, 2020). Widespread expression of the NR2B subunit has been observed in parts of the cerebral cortex responsible for pain behavior including the ACC and the insular cortex (Wei et al., 2001). The majority of NMDA receptor-mediated currents in the ACC are due to receptors having NR2A and/or NR2B subunits. This observation was further refined by a study that reported the decrease in NMDA receptor-mediated LTP in the ACC by the administration of NR2B antagonists. It is noteworthy that even though the LTP was decreased, it was not completely attenuated (Zhao et al., 2005). LTP in the insular cortex also depends on the NMDA receptors containing both NR2A and/or NR2B subunits. This was confirmed by the observation that NMDA antagonists prevented the development of LTP in IC due to tetanic stimulation in rats (Liu et al., 2013). Suppression of NR2B subunit and its downstream pathway (CREB) in spinal dorsal horn accounts for the attenuation of circadian pain in nerve-injured rats (Xia et al., 2016). Moreover, spinal NR2B tyrosine phosphorylation is reported to contribute to the central sensitization and synaptic plasticity in neuropathic pain and chronic migraine (X.-Y. Wang et al., 2018). Thus, it is evident that the NR2B subunit plays an important role in NMDAR mediated central sensitization and maintenance of CINP.

One notable area of exploration is the use of natural compounds in alleviating chronic pain, including conditions like neuropathic pain and inflammatory pain. Many of these compounds demonstrate anti-inflammatory and antioxidant effects, targeting pathways involved in pain signaling and modulation. For instance, curcumin, derived from turmeric, has shown anti-inflammatory properties and the ability to modulate pain-related molecular pathways (X. Zhang et al., 2020). Similarly, cannabinoids from

the cannabis plant have gained attention for their potential in pain relief, interacting with the endocannabinoid system.

1.8 Animal models for CINP

Developing rodent models of CINP that faithfully recapitulates the diverse symptoms reported by patients poses a considerable challenge. CINP symptoms such as numbness, tingling, and ongoing pain rely on verbal communication from patients, making it difficult to directly translate these experiences to animal models. Consequently, many studies have focused on assessing evoked pain-like behaviors, a common approach in various chronic pain models. Recent investigations into novel measures of spontaneous pain in rats with CIPN have identified deficits in burrowing behavior and voluntary wheel running following the administration of paclitaxel, as presented at the 6th International Congress on Neuropathic Pain (NeuPSIG 2017). Rodent models of CIPN have been established using different chemotherapeutic agents, including paclitaxel, docetaxel, vincristine, cisplatin, oxaliplatin, and bortezomib.

Several studies examining paclitaxel-induced neurotoxicity involved the direct application of paclitaxel to peripheral nerves, resulting in microtubule degeneration and specific aggregation (Höke and Ray, 2014). However, the relevance of such local applications to understanding CINP mechanisms induced by systemic administration is limited due to the high endoneurial concentration (Polomano et al., 2001). Subsequent studies developed rodent models of paclitaxel-induced painful neuropathy through systemic administration, either intravenously or intraperitoneally, often utilizing intermittent dosing regimens to mimic chemotherapy cycles. Most CINP models typically involve the sole administration of a specific chemotherapeutic agent,

excluding the presence of a tumor load. However, some reports describe a rat model with an implanted subcutaneous tumor receiving monotherapy including paclitaxel and cisplatin treatment (Alexandre et al., 2006; S. Zhang et al., 2019). Ethical considerations and the practical feasibility of maintaining animal health must be paramount, as pain-like behaviors cannot be accurately assessed in rodents suffering from systemic toxicity, rendering them lethargic and unresponsive to stimuli. While models with a tumor may be deemed more clinically relevant, the practical and ethical challenges associated with this approach should not be underestimated. Considering that chemotherapy is often administered post-surgery to eliminate micro-metastases, modeling CIPN through chemotherapy alone remains a valid and widely adopted approach. Encouraging the use of established intermittent dosing schedules across laboratories can enhance reproducibility and facilitate a deeper understanding of causal mechanisms underlying CIPN. Another, approach would be combination-based chemotherapy induced neuropathic pain, observed in the patients who have suffered from the CINP after receiving a combination of the anticancer drugs.

1.9 Drug failures stemming from preclinical model incompatibility

Despite a substantial increase in understanding of CINP pathophysiology, no FDA-approved or effective treatments are available for its management. Developing a more clinically relevant animal model could significantly enhance the research output for CINP treatment. Despite vast investigation on drug development for CINP, the overall success rate of drug translation to the clinical setup remains low due to several factors. One such factor is the lack of animal models which can mimic the clinical setup of chemotherapy treatment and correlate the outcome to bridge the translational gap. Paclitaxel/platinum/vincristine combinations delivered significant outcomes in phase

III clinical trials and are now considered standard therapy for advanced carcinomas including breast cancer and gastrointestinal cancers (Höke and Ray, 2014; Ozols, 2000). We used behavioral and pharmacological tools to develop and validate a clinically relevant, combination-based chemotherapeutic cocktail model of peripheral neuropathy. Several models are already in use for CINP research, such as paclitaxel (PTX) (Flatters et al., 2017a), vincristine (VCR) (Montague and Malcangio, 2017), oxaliplatin (OXL) (King et al., 2017), etc. which has made tremendous progress in unraveling the pathophysiology of CINP. But the preclinical investigations are lacking their translational values and one of the reasons being cited by researchers is the unavailability of models which can best represent the clinical condition of CINP. Based on the drug prescription record for cancer survival as well as recent literature on clinical studies, it is suggested that chemotherapeutics agents are generally prescribed in a combination of two or three drugs (Flatters et al., 2017b). Numerous studies documented that the combined chemotherapy regimen of vincristine, cisplatin and paclitaxel is the most prescribed treatment for cancers like ovarian, breast and hematological malignancies (Hao et al., 2019; Ozols, 2000; Pentheroudakis et al., 2006; Toma et al., 2017; Tu et al., 1995; Ueda et al., 2010). Therefore, in the present study, we used a standard animal model development paradigm using behavioral, pharmacological and molecular tools to develop a rationalized combination-based rat model of CINP. The three-step strategy was acquired to develop and validate the novel animal model; 1) Face validity: disease phenotypes such as signs, symptoms and clinical observations 2) Predictive validity: robustness of standard therapeutic outcomes; 3) Constructive validity: similarity in the biological changes between human and animal. The present study may bridge the gaps between bench to bedside and

provide a better alternative to test the compounds and dissect the pathophysiology of CINP.

1.10 Bergenin: A potential therapeutic breakthrough for treatment of chronic pain

Bergenin is a C-glucoside of 4-O-methyl gallic acid found naturally in a diverse range of plant species. Various studies have demonstrated the anti-inflammatory potential (Tang et al., 2021), anti-tussive (Pu et al., 2002), anti-HIV (Suksungworn et al., 2021), anti-allodynic (Villarreal et al., 2020), and neuroprotective properties (Barai et al., 2019) of Bergenin. In the present study, we have investigated the effect of Bergenin in animal model of chemotherapy-induced neuropathic pain and dissected the detailed mechanism of action of Bergenin involving modulation of TRP channels-mediated NR2B signalling in DRG and spinal cord of neuropathic rats. Previous studies suggested that Bergenin ameliorates inflammatory pain via downregulating phosphorylated mitogen-activated protein kinases (p38-MAPK) and CDK5 expression in LPS-treated rats (Xiang et al., 2020). The activation of p38-MAPK and CDK5 results in the upregulation of TRPA1/TRPV1 and TRPM8 in the peripheral somatosensory system (Yang et al., 2022). The chemical structure of Bergenin is given in Figure 1.8.

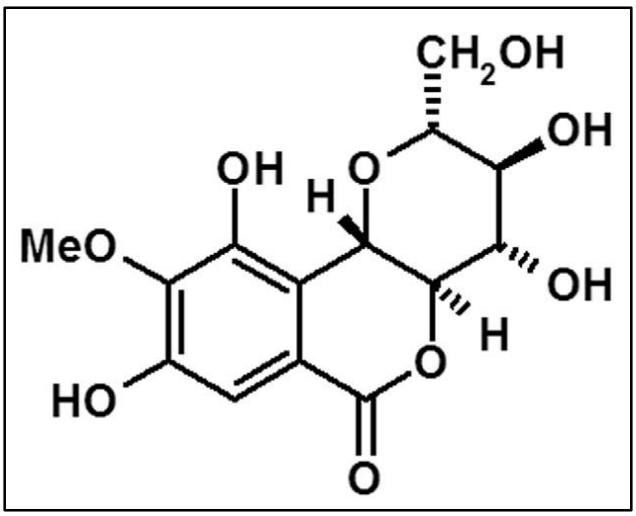

Figure 1.8 Chemical structure of Bergenin

1.11 siRNA as a promising approach for the treatment of CINP

Recent advancements in molecular medicine have led to the exploration of small interfering RNA (siRNA) as a promising therapeutic approach for CINP. siRNA is a class of short RNA molecules that can selectively silence target genes by interfering with their expression. This precision makes siRNA therapy an attractive option for addressing the specific molecular pathways involved in neuropathic pain.

1.11.1 siRNA structure and function: a brief overview

Over the past decades, we have observed a strong relationship between biological research and biomedical researchers involved in developing innovative methods for treating, preventing, and managing pain (Bidve et al., 2020; de Fougerolles et al., 2007). RNA interference (RNAi), allows the knockdown of specific genes for the treatment of CINP (Zhang and Jeske, 2020). Anatomically, siRNA is a group of non-coding, double-stranded RNA molecules that comprise 21–23 nucleotides in each strand (Weng et al., 2019). Generally, dsRNA processed by a specialized ribonuclease (RNase) III-like enzymes namely Dicer into the cytosol system. The endonuclease

argonaute 2 (AGO2) component of the RNA-induced silencing complex (RISC) in the cytoplasm, the RISC cleaves the sense and antisense strand. Subsequently, siRNA senses strain experiences, cleavage, and expulsion, while the antisense strand of the siRNA is thermodynamically targeted to the complementary messenger RNA (mRNA) (Chen et al., 2018a). Partial hybridization of antisense siRNA with target mRNA results in inhibition of translation, while absolute complementary hybridization is associated with mRNA degradation (Miller et al., 2005). The US Food and Drug Administration (FDA) approved patisiran (Onpattro; Alnylam Pharmaceuticals), a siRNA that acts on the liver, for the treatment of hereditary transthyretin amyloidosis (hATTR) with polyneuropathy on August 10, 2018 (Zhang et al., 2021). Patisiran's approval gives patients a new hope with hATTR and ushers a new era in the RNAi therapy field (X. Zhang et al., 2019). SYL1001, a siRNA targeting TRPV1 has been translated into clinics for the treatment of ocular pain and dry eye condition (Moreno-Montañés et al., 2018). Over the next five years, breakthrough treatments could be enabled by newly discovered RNAi, advanced RNAi payloads with increased selectivity and potency, and improved mechanisms for systemic and local RNAi delivery.

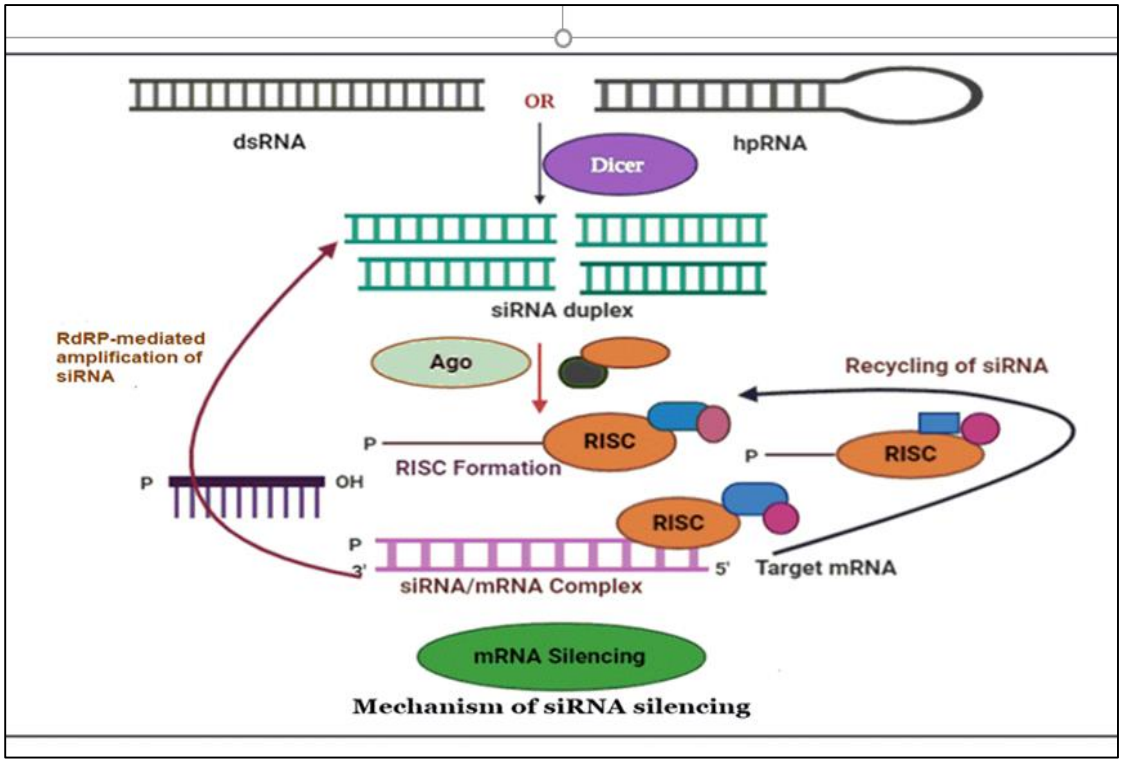

Figure 1.9 Silencing of the siRNA-regulated gene in eukaryotes: Double-stranded RNAs or hairpin RNAs (hpRNAs) generate small duplexes of siRNA through the activity of the Dicer gene. The RNA strand guide binds to Argonaute (Ago) and other proteins to form an RNA-triggered silencing complex (RISC). The siRNA/ RISC complex then binds to the target mRNA complement sequence. Reprinted with permission by Elsevier from own source reference (Akhilesh et al., 2022).

1.12 Collecting evidence supporting TRPA1 channels as a potential therapeutic target for CINP

RNAi has increasingly become the most frequently used gene knockdown mechanism because of its higher affinity, specificity, and potency (Sivakumar et al., 2019). RNAi is a highly evolved mechanism that silences gene expression by targeting mRNA (Chernikov et al., 2019). Over the last decade, hundreds of molecular targets have been recognized for their roles in pain regulation. With small molecules, nevertheless, most molecular targets are not readily druggable. A therapeutic approach referring to these non-drug targets is RNAi. The number of studies using small

interference RNAs (siRNAs) to track new pain management targets is rising significantly. Numerous studies have already documented the use of siRNA strategies to investigate the function of the TRPA1 receptor. (Benitez-Del-Castillo et al., 2016; Liu et al., 2018; Ning et al., 2022). Kasama, S and his colleagues reported their research of paratracheal administration of TRPA1 antagonist which inhibited the upregulation of TRPA1 in DRG and spinal cord and eradicated CFA-induced inflammatory and chemotherapy-induced thermal hyperalgesia and mechanical allodynia (Kasama et al., 2007). Overexpression of TRPA1 in CINP is a potent inducer of nerve excitotoxicity and it increases the action potential and hypersensitization of nerve conduction (Ikeda-Miyagawa et al., 2015). Subsequently, a second siRNA targeted to a different region of the TRPA1 gene was employed and confirmed the antinociceptive action of a TRPA1 knock-down. Besides the TRPA1 antagonists, TRPA1 siRNAs also have a potential role in the treatment of the neuropathic pain (Trevisan et al., 2013). Molecular studies such as immunohistochemical analysis and western blot/RT-PCR confirmed that TRPA1-siRNA directly reduced TRPA1 protein expression in the superficial dorsal horn of the spinal cord following nerve injury in rats (Qin et al., 2008). Intriguingly, they indicated that the expression of TRPA1 mRNA was upregulated using PTX administration but downregulated in knockout mice. Cisplatin-induced changes in small DRG neurons were reduced significantly in TRPA1 in transgenic mice (Sánchez et al., 2023). The treatment with PTX increased the expression of TRPA1 protein in the spinal cord, with higher protein levels in the superficial layers. Intrathecal injection of siRNAs using adeno-associated virus serotype 9 (AAV9) vector inhibits the DRG and spinal cord expression of target genes for an extended period, suggesting that this approach may be used to regulate the expression of genes associated with CINP (S.

Zhang et al., 2019). Possible mechanism of TRPA1 in the CINP was showed in the Figure 1.10.

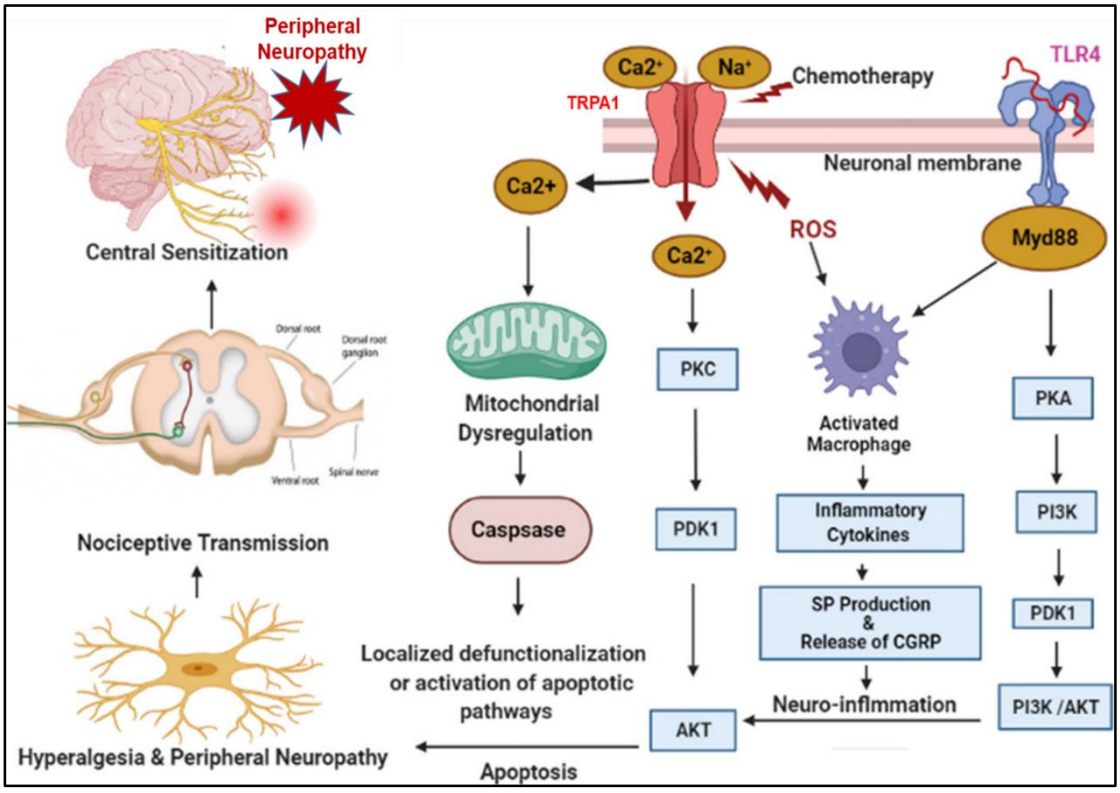

Figure 1.10 TRPA1 as promising target in chemotherapy-induced neuropathic pain: Reproduced with permission from Elsevier form own reference sources (Akhilesh et al., 2022).

1.13 Current status of siRNA interventions as a near-future therapeutic tool

siRNA is used as a potential tool for understanding the physiological and pathological functions of various genes (Weng et al., 2019). Long-term stability of the 5-end transmission sensor strand (SS) prohibits their entrance via siRNA as integral components of highly complex gene networks functioning by arresting their targets and attempting to have strong effects on the RNA-induced silencing complex (RISC), thereby providing a definite mechanism of action (Neumeier and Meister, 2021). The low stability of the 50% antisense strands (AS) helps facilitate the segregation from the

SS and therefore its entry into the RISC, promoting RISC-AS mediated cleavage of target mRNA (Neumeier and Meister, 2021). Thus, extensive knowledge of siRNA and its transfection will help us understand the function of gene networks better and develop effective and safe methodologies for genetic manipulation. Consequently, the effectiveness of siRNA as a treatment approach is based on the transfection process (Masumoto et al., 2013). Furthermore, it should also be highlighted that gene expression is knocked down rather than knocked out by siRNA. The first move towards using siRNAs in therapeutics is to design a unique siRNA sequence accessible for multiple algorithms. Following the designing of desired siRNAs sequences, they can be produced or synthesized chemically to be integrated into the cells for expressing the genes. The latter is transcribed from transcription structures inside the cell (such as plasmids and viral carriers) that transmit the short-hairpin siRNA precursors (shRNA) (S. Zhang et al., 2019). By using chemical synthesis, the quantity and purity of the siRNA can be controlled and stability can also be improved, which are essential tools required for delivery purposes. Additionally, siRNA is inserted into the cell and then the cell's intrinsic RNAi machinery initiates and executes the gene silencing process through the duplex siRNA. Another strand namely the antisense strand which is loaded into a kind of protein complex known as the RNA-induced silencing complex (RISC), can act as the template that has a crucial role in recognizing complementary mRNA (Neumeier and Meister, 2021). The benefit of the targeted sequencing followed by siRNAs in contrast to drug therapies is that it has a high level of efficiency and low toxicity as well as low side effects (Shen et al., 2018). The silencing process schematics for the siRNA are given in Figure 1.9. With the potential to easily inhibit a specific pathogenic molecular and cellular pathway, there was a lot of excitement about the

clinical application of siRNAs in certain gene-related disorders such as cancer infections and neuropathy, as well as autoimmune illnesses (Chen et al., 2018b; Liu et al., 2007). Their convenience of personalization and maximum demand lead to signify the promise of personalized medicine. Several studies have been conducted in the last decade to investigate the therapeutic potential of siRNAs in both in-vitro and in-vivo experimental settings. Furthermore, because of the lack of an effective delivery method, practical translation of siRNA therapy has been difficult (Chen et al., 2018b).

1.14 Current challenges and future prospects: a roadmap for advancement of siRNA therapeutics

Systemic administration of naked siRNA is deteriorated by serum nucleases, disposed of by defensive immune cells, and eliminated by renal filtration (Miller et al., 2005; Shen et al., 2018). The cell membrane contains a negative charge while naked siRNA is also negatively charged at the normal pH so they repel, which imposes a challenge to their cellular delivery (Cooper et al., 2014). The hydrophilic nature of siRNA makes it difficult for it to enter cells by passive diffusion. Other issues involve poor tissue penetration, inconsistency, limited efficiency to internalize, and unspecified immune stimulation (Miller et al., 2005). The lifespan of a naked siRNA is only a few minutes to a few hours in-vivo and with the help of nano-engineering and bio-conjugates reactions increased the duration and potency. A study has reported the cellular uptake percentage was 0.7 % of the injected dose. As a result, naked siRNAs get destroyed before reaching their target cell. Hence, there is a need for a delivery system that would not only protect the SiRNA molecules across systemic delivery but also maximize the delivery into the target cell. Recent research on siRNAs as cancer therapies is focused on intravenous delivery of the intervention to avoid exposure to

various catalytic enzymes (Mukherjea et al., 2008). Other defined pathways include local delivery via intraocular and intra-tumoral injection and local delivery to the CNS (Bholakant et al., 2020; Naik et al., 2021). The current evidence suggests that novel strategic delivery tools must be employed to improve the cellular uptake of siRNA to enhance their clinical utility.

1.15 Nano-carriers as promising candidates for siRNA delivery

The siRNA molecules are administered by local and systemic routes for in-vivo efficacy measurement purposes. However, several studies rely on the use of relatively large quantities of siRNAs, but at these concentrations, intracellular immune responses could be activated (Benitez-Del-Castillo et al., 2016). Although this framework is invasive and constrained to tissues that are adequately reachable in systemic application aspects. Several studies reported hydrodynamic transfection of siRNAs, i.e., the rapid (20sec) high-pressure injection of a siRNA-containing solution (up to 2 ml). The hydrodynamic infusion has spurred the effective induction of RNAi to the kidney, liver, lung, pancreas, and spleen, which is likely due to a transient increase in membrane-permeability (Ahmadzada et al., 2018). Recently, attention has been focused on the use of nanotechnology as a novel siRNA delivery tool, including different approaches such as the use of liposomes, exosomes, polyethyleneimine, and bio-conjugation, etc. Nano-drug carriers could encapsulate hydrophilic drugs at their aqueous component whereas, their phospholipids bilayer localize the lipophilic drugs. They possess the properties of nano-scale, similar biofilm structure, and excellent biocompatibility and are becoming more and more useful in drug development as a delivery system. Due to substantial advances in nanoengineering, a variety of medicinal formulations are safe for clinical use and several products undergo several clinical trials (Dermani et al., 2019; Salzano

et al., 2015; Young et al., 2016). Viruses or synthetic agents are used as delivery vehicles in current siRNA transfer approaches. To make siRNA therapy a reality, these delivery vehicles must be replaced with low toxicity and high target specificity approaches. Nanoparticles are a novel and promising delivery mechanism for siRNA therapy because they have the inherent capacity to cross biological barriers and naturally carry functional siRNAs across cells (Sarett et al., 2017). Because nanoparticles are natural nanocarriers produced from endogenous cells, they will be better tolerated by the immune system as therapeutic delivery agents. Nanoparticles made from genetically modified cells can also deliver tiny RNAs to specific tissues and cells. As a result, nanoparticle-based siRNA delivery may offer an untapped, effective delivery technique to overcome constraints like inefficiency, non-specificity, and immunogenic responses. Another method, such as polyethyleneimine (PEIs) could be used. The well-established PEIs are synthetic linear or branched polymers that are difficult to access across a wide range of molecular weights important to the siRNA delivery strategy (Höbel and Aigner, 2010). Ascribed to the prevalence of a protonable amino group in each third position, which leads to a high cationic charge density at physiological pH. PEIs can form noncovalent DNA clusters as well as small RNA molecules like siRNAs (Höbel and Aigner, 2010) . Nano-carriers, particularly liposomal formulations, emerge as highly promising vehicles for the delivery of siRNA, holding significant potential for addressing chronic pain at the genetic level. Liposomes, composed of lipid bilayers, serve as effective carriers by encapsulating and safeguarding siRNA molecules, shielding them from degradation in the bloodstream and enabling targeted delivery to specific cells or tissues. This liposomal structure provides a biocompatible and biodegradable environment, ensuring controlled release

of siRNA payloads while minimizing off-target effects. The adaptability of liposomal surfaces allows for enhanced cellular uptake and efficient endosomal escape, overcoming challenges associated with traditional siRNA delivery methods. These attributes position liposomal nano-carriers as innovative tools for precise and efficient siRNA delivery in the treatment of chronic pain and other diseases, offering a promising avenue for targeted gene silencing and therapeutic intervention.