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Ion channels and neuronal hyperexcitability in chemotherapy-induced peripheral neuropathy: Cause and effect?

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Abstract

Cancer is the second leading cause of death worldwide and is a major global health burden. Significant improvements in survival have been achieved, due in part to advances in adjuvant antineoplastic chemotherapy. The most commonly used antineoplastics belong to the taxane, platinum, and vinca alkaloid families. While beneficial, these agents are frequently accompanied by severe side effects, including chemotherapy-induced peripheral neuropathy (CPIN). While CPIN affects both motor and sensory systems, the majority of symptoms are sensory, with pain, tingling, and numbness being the predominant complaints. CPIN not only decreases the quality of life of cancer survivors but also can lead to discontinuation of treatment, thereby adversely affecting survival. Consequently, minimizing the incidence or severity of CPIN is highly desirable, but strategies to prevent and/or treat CIPN have proven elusive. One difficulty in achieving this goal arises from the fact that the molecular and cellular mechanisms that produce CPIN are not fully known; however, one common mechanism appears to be changes in ion channel expression in primary afferent sensory neurons. The processes that underlie chemotherapy-induced changes in ion channel expression and function are poorly understood. Not all antineoplastic agents directly affect ion channel function, suggesting additional pathways may contribute to the development of CPIN Indeed, there are indications that these drugs may mediate their effects through cellular signaling pathways including second messengers and inflammatory cytokines. Here, we focus on ion channelopathies as causal mechanisms for CPIN and review the data from both pre-clinical animal models and from human studies with the aim of facilitating the development of appropriate strategies to prevent and/or treat CPIN.

Keywords

Cancer, chemotherapy-induced peripheral neuropathy, neuropathic pain, ion channel, antineoplastic, taxane, platinum, vinca alkaloid

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Introduction

Cancer (all sites) presents an enormous global disease burden. In 2015, 17.5 million new cases were diagnosed, and there were 8.7 million cancer deaths. The lifetime risk of developing cancer is one in three for men and one in four for women. In men, prostate cancer was the most common, while cancers of the trachea, bronchi, and lung were the leading causes of cancer death. In women, breast cancer was the most common cancer as well as the leading cause of cancer deaths.[1] Treatment options are tumor and patient specific, and routinely include, alone or in combination, surgery, radiation therapy, immunomodulatory therapy, and adjuvant antineoplastic chemotherapy. Among the antineoplastics commonly encountered in the aforementioned settings are those belonging to the taxane, platinum, and vinca alkaloid families.[2–6] Although efficacious, these agents are associated with significant, debilitating, adverse side effects, including chemotherapy-induced peripheral neuropathy (CIPN). As cancer survival rates have

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improved,[1,7] the burden of CIPN has correspondingly increased. CIPN can affect both sensory and motor systems and has a profound negative impact on long-term quality of life (QoL).[8–11] There is evidence that patients deem CIPN to be so unacceptable that they are willing to discontinue treatment despite the potential for a decrease in survival.[10]

The symptoms of CIPN are mainly sensory, with pain (including hypersensitivity to cool temperatures), tingling, and numbness (especially in the hands and feet), being the predominant complaints.[9–11] The distal distribution of the sensory complaints reflects the fact that large sensory nerve fibers are most commonly affected. In fact, dorsal root ganglion (DRG) neurons are a prominent target, possibly due to the fact that they are less protected by the blood–brain barrier.[8,11] Motor symptoms are also encountered and include weakness, cramps, and gait dysfunction; sweating abnormalities, constipation, and light-headedness are evidence of autonomic symptoms.[8,9]

The symptoms of CIPN can be clinically assessed and graded. While there are several grading scales (including specific chemotherapy symptom scales), the two commonly employed scales are: (1) The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE or just NCI) and (2) The Total Neuropathy Score. Both scales are graded from 0 to 4 for each effect measured, with 4 indicating the greatest degree of severity. The NCI scale has a limited scoring range (0–4) because it only takes into account sensory effects and requires significant training to obtain high user reliability; in contrast, the Total Neuropathy Score scale has a wider scoring range (0–32 in total) as it includes sensory symptoms, motor symptoms, pin sensitivity, vibration sensitivity, strength, deep tendon reflexes, and neurophysiological effects from the sural and peroneal nerve and is considered reliable, having a high inter-observer reliability.[11,12]

The lack of a standardized approach to assess or grade CIPN can lead to variability in determining the prevalence of CIPN.[11,13] Depending on the drug regimen, the overall rate of CIPN ranges from 19% to 85%.[13,14] The incidence rate, however, is time sensitive; Seretny et al.[13] compiled data from 31 different studies and found that within the first month following chemotherapy treatment, ~68% of patients had CIPN, by three months, 60% still had CIPN, and after six months, 30% were still affected. Another reason for the variability in the rate of occurrence may be due to differences in the chemotherapeutics themselves. As noted above, the three main chemotherapeutic classes commonly associated with CIPN are as follows: taxanes, platinum-based agents, and vinca alkaloids. The taxanes, which include paclitaxel, docetaxel, and cabazitaxel, are used to treat breast, ovary, non-small cell lung, gastric, head and neck, and prostate cancers. In contrast, platinum drugs, including cisplatin, carboplatin, and oxaliplatin, are used to treat cancers localized to the testes, ovary, cervix, uterus, head and neck, colon, and prostate. Finally, the vinca alkaloids, which include vincristine, vinblastine, vindesine, and vinorelbine, target hematologic cancers and pediatric sarcomas. Each antineoplastic class has a different mechanism of action and dose/rate of CIPN occurrence; it is therefore crucial to assess each one individually in order to reveal unique and/or common mechanisms involved in the pathogenesis of CIPN. One common mechanism to all three antineoplastic classes is changes in ion channel expression in primary afferent sensory neurons. In this review, we focus on this common pathway and review data from pre-clinical animal models as well as data from human studies where such exists. By better understanding common pathways, it may be possible to develop appropriate strategies to treat CIPN, or even better, prevent its onset.

Taxanes

Taxanes exert their effect by preventing the depolymerization of microtubules, leading to both apoptosis and the inhibition of axonal protein transport, thereby altering the function of distal sensory axons, either or both of which can result in CIPN.[8,9,15] The incidence of CIPN can vary depending on the dose *per* cycle, duration of infusion, cumulative dose, and treatment schedule, with paclitaxel more likely than docetaxel to cause CIPN.[10,11,16]

Paclitaxel

The incidence of paclitaxel-induced CIPN varies as a consequence of a number of factors: (1) the cumulative dose, the total dose at which CIPN symptoms first appear is $>300\,mg/m^2$, while a dose between 1400 and $1500\,mg/m^2$ has been linked to Grade 3 neuropathy; (2) rapid rate of infusion, there is increased neuropathy with a 3 h versus 24 h infusion duration; and (3) increased single dose, symptoms can start 24 to 72 h after administration of a single (high) dose of $250\,mg/m^2$ but usually occurs after multiple doses of the conventional dose of $<200\,mg/m^2$.[10,11,16,17]

Paclitaxel can cause an acute pain syndrome that develops one to four days after initiating chemotherapy and is characterized by myalgia and arthralgia.[11] This acute pain syndrome is predictive of future development and severity of paclitaxel-induced CIPN.[11,17] While mild symptoms can improve with reduction in dose, paclitaxel-induced neuropathy can persist for months to years.[10] In the short term (i.e., 12 months), ~80% of breast cancer patients treated with paclitaxel

developed numbness in the hands and feet[18]; the duration and incidence of CIPN may also be influenced by the specific cancer under treatment as Pignata et al.[19] reported that in patients with ovarian cancer, the probability of having CIPN after six months was 15% and 11% after two years. Alternatively, the differences in the incidence rates could also reflect drug-drug interactions as patients in the Pignata study received both carboplatin and paclitaxel. On average, while 50% of patients with paclitaxel-induced CIPN show recovery after nine months, roughly 40% of patients still display symptoms after three years.[8,10,11]

Docetaxel

Docetaxel-associated CIPN occurs at cumulative doses of $>100\,mg/m^2$, is milder than that associated with paclitaxel, and can resolve spontaneously following cessation of therapy.[10,16] In contrast to paclitaxel, Grade 3/4 neuropathy occurs in <10% of patients, but is proportional to cumulative dose.[10,16] While it may not be as severe as paclitaxel, up to one-third of patients treated with docetaxel will have CIPN that persists anywhere from 3 to 13 years after completing treatment.[20,21] Therefore, even ''mild'' to ''moderate'' CIPN can still affect a large number of patients for extended periods of time and further underscores the need for effective treatments and/or protective strategies.

Platinum compounds

Platinum compounds, including carboplatin, cisplatin, and oxaliplatin, form platinum adducts that promote cross linking that can alter nuclear DNA structure and synthesis,[22,23] as well as mitochondrial DNA, leading to oxidative stress.[9,17] As a class, they contribute to the development of CIPN by impairing the electrophysiologic function of DRG neurons as demonstrated by a reduction and/or loss of the sensory action potential in nerve conduction studies.[9] Compared to cisplatin and oxaliplatin, carboplatin-induced CIPN is less severe and less common, occurring in 4% to 6% of patients.[10,24]

Cisplatin

The risk of developing cisplatin-induced CIPN increases with cumulative dose and higher single dose administration.[8,11,25] The cumulative dose associated with risk of neurotoxicity is $>350\,mg/m^2$.[26] A common experience with cisplatin is the phenomenon known as ''coasting,'' wherein CIPN symptoms can worsen or start *after* completion of therapy.[10] Recovery is quite prolonged and is often incomplete, largely due to the fact that the platinum adducts can persist in body tissues for years.[27] When patient serum was tested 20 years after treatment, platinum levels were still elevated and the levels were associated with the severity of neuropathy.[28] Thus, it is not surprising that studies have found patients suffering from CIPN long after their treatment has ended. One such study found 20% of patients treated with cisplatin had persistent sensory neuropathy,[26] while another reported that overall, 80% of patients had demonstrable nerve damage, with 28% of patients remaining symptomatic 15 years after treatment, with 6% having severe, disabling CIPN.[9,11,29]

Oxaliplatin

The incidence of CIPN after oxaliplatin treatment also increases with cumulative dose, single dose, and rapid infusion rate.[11,30,31] Severe neuropathy occurs in 10% of patients after nine cycles of chemotherapy and up to 50% of patients after 14 cycles.[32] The cumulative dose associated with peripheral neuropathy is $>550\,mg/m^2$,[9,11,15] and with a cumulative dose of 750 to $850\,mg/m^2$, 82% to 93% of patients experience some form of CIPN, with 12% to 34% of patients experiencing severe (Grade 3/4) neuropathy.[10,11,32,33]

Oxaliplatin-induced neuropathy is characterized by two distinct syndromes. First, there is the acute syndrome (which appears to be unique to oxaliplatin among the platinum compounds), which occurs during or immediately after administration of oxaliplatin, is seen in the majority of patients (85%–95%), can be triggered by cold, is characterized by neuronal hyperexcitability, and can resolve in hours or days after onset.[34–37] Second, there is a chronic syndrome, which is comparable to the neuropathy seen with cisplatin and other platinum compounds where ''coasting'' occurs.[9,35] This chronic syndrome can affect 80% to 90% of patients, with 40% to 50% of patients experiencing at least Grade 2 symptoms, while 10% to 20% had Grade 3 or 4 symptoms.[10,35,38] Although oxaliplatin-induced CIPN is largely reversible with an average time of recovery of 13 weeks, some patients experience persistent neuropathy.[11] It is important to note that what patients report (i.e., what is their subjective experience of their disease) is not necessarily the same as that which is ''objectively'' measured clinically. Objective assessment, such as with doctor scored NCI assessment or via nerve conduction studies, indicates that lasting abnormalities and decreased sensory amplitudes can be detected in 10% to 75% of patients up to six years after treatment is terminated.[9,31,39,40] But this is not necessarily a foolproof diagnostic tool. Thus, one study reported that on *clinical* assessment with the NCI scale, 10% of patients will have objective measures consistent with a diagnosis of CIPN two years after treatment has ended even

though 60% of patients will *report* symptoms consistent with lasting neuropathy when asked to describe how they are feeling[41]; elsewhere, patients have reported significant symptoms up to 11 years after treatment ended,[42] again indicating that clinical assessment does not fully account for what the patient is experiencing. Conversely, there are instances where the incidence of patient reports of neuropathic symptoms is less than that as evidenced by altered nerve conduction studies; in that same study by Bennett et al.,[41] while 60% of patients reported lasting neuropathy symptoms, 85% of patients demonstrated signs of neuropathy as measured via nerve conduction studies, suggesting that some patients with chronic symptoms may learn to "adapt" to their sensory abnormalities.[11] Such discordance demonstrates the need for improving our clinical assessment tools and/or the need to more adequately account for a patient's subjective experience when diagnosing CIPN.

Vinca alkaloids

Vincristine, one of the vinca alkaloids, is among the more toxic chemotherapy agents, and nearly all patients treated with vincristine develop some degree of neuropathy.[9,10] Vincristine produces a loss of microtubules by preventing tubulin polymerization, which results in impaired axonal transport.[9,10] Patients with vincristine-induced neuropathy have reduced amplitudes of both motor and sensory action potentials as well as slightly reduced conduction velocities.[9] The toxicity due to vincristine is both cumulative and dose dependent, with a dose-related threshold of $>4\,mg/m^2$ and severe neuropathy occurring after a cumulative dose of 15 to 20 mg/m^2.[9–11,43,44]

Similar to cisplatin, "coasting" is common to patients treated with vincristine; it is seen in approximately 30% of patients, and the most common symptoms experienced are numbness and tingling in the hands and feet, which are seen in 35% to 45% of patients.[9–11,43,44] At least one study has suggested that vincristine-induced CIPN is largely reversible with good long-term prognosis,[10] whereas others studies have found that vincristine-induced CIPN persists for extended periods of time.[11] Mild sensory symptoms were seen in 32% of non-Hodgkin lymphoma survivors 34 months after the end of treatment, while 14% still had symptoms after nine years.[11,45] In addition, 30% of children with acute lymphoblastic leukemia still had CIPN symptoms seven years after the end of treatment.[46] These studies are a reminder that while the prognosis (in terms of survival) may be good for the majority of vincristine-induced neuropathy patients, there are still many that suffer with a diminished long-term QoL and who need effective treatment for their neuropathy.

Despite the fact that distinct chemotherapeutic agents have different modes of action, many produce a long-lasting peripheral neuropathy, which has significant public health ramifications. No uniformly effective treatments for CIPN exist, indicating that there is a pressing need to develop effective therapeutic strategies. There is increasing evidence that pain, both physiologic and pathologic, is associated with ion channel regulation and/or modulation to regulate neuronal activity in the peripheral nervous system.[47] Therefore, a clear understanding of how the functional expression of major neuronal ionic channels is altered by chemotherapy-related drugs will ideally reveal novel therapeutic targets that will help prevent or treat CIPN (Tables 1 and 2). It is also possible that chemotherapy drugs do not act directly on ion channels, and second messengers such as cytokines could provide a link between the two as they are already implicated in pain[82,83] and ion channel regulation.[84,85] Therefore, the aim of this article is to review current research in the context of neuronal ion channels and pathways that are modulated by chemotherapeutic agents with the hope that it will reveal new and unappreciated research areas that warrant further investigation.

Neuronal excitation

Action potentials (APs) can encode information in their firing frequency and pattern, and perpetuate signal transmission as they propagate down the nerve axon and invade the axon terminal, where the resulting membrane depolarization initiates the sequence of steps necessary for vesicle mobilization, fusion, and exocytosis. APs are generated by the activation of ion channels. There are three main phases of the AP: (1) the depolarization phase—which consists of the upstroke and overshoot where the membrane potential becomes positive, (2) the repolarization phase—which consists of the downstroke after the depolarization phase and returns the cell's membrane potential to a negative potential (resting membrane potential), and (3) the after-hyperpolarization phase (AHP)—which is when the membrane potential falls below the resting membrane potential before returning to the resting state. Different ion channels are responsible for generating and sustaining the different phases.[86] Voltage-gated Na^+ channels mediate the depolarization phase, voltage-gated Ca^{2+} and K^+ channels mediate the repolarization phase, K^+ channels are responsible for the AHP, and K^+, Na^+, and HCN channels are responsible for setting the resting membrane potential, all of which help control the excitability of neurons.[86] We will briefly discuss each of these ion channel families, as well as Transient Receptor Potential (TRP) channels, and their role in CIPN.

Table 1. Ion channel modulation by antineoplastic agents.

Antineoplastic agent	Na^+ Channels	K^+ Channels	Ca^{2+} Channels	TRP channels
Taxanes				
Paclitaxel	↑ $Na_V1.7$[48]	↑ $K_V1.2$[48] ↑ $K_V11.3$[48] ↑ $K_{ir}3.1$[48] ↑ HCN1[48] ↓ $K_{ir}1.1$[48] ↓ $K_{2P}1.1$[48] ↓ $K_{ir}3.4$[48]	↑ $\alpha_2\delta$[49–52] ↑ $Ca_V3.2$[54]	↑ TRPV1[53]
Platinum compounds				
Cisplatin			↑ N-type[55] ↑ $\alpha_2\delta$[55] ↓ L-type[55] ↓ T-type[55] ↓ P/Q-type[55]	
Oxaliplatin	↑ $Na_V1.6$[56] ↑ $Na_V1.4$[61] ↑ $Na_V1.7$[63] ↑ $Na_V1.8$[61,62] ↑ $Na_V1.9$[65] ↓ $Na_V1.5$[66]	↑ $K_{ir}3.1$[57] ↑ HCN1[62] ↓ TREK1[62] ↓ TREK2[64] ↓ TRAAK[62] ↓ KCNQ[67,68] ↓ $K_V1.1$[62]	↑ $\alpha_2\delta$[50]	↑ TRPM8[58–60]
Vinca Alkaloids				
Vincristine			↔ $\alpha_2\delta$[50]	

Note: ↑: upregulation; ↓: downregulation; ↔: no change; TRP: transient receptor potential.
Changes in mRNA, protein or currents, refer to original paper for details.

Sodium channels

Biophysics

Voltage-gated Na^+ (Na_V) channels are one of the more prominent ion channels associated with CIPN. The first indications of their involvement come from studies where administration of oxaliplatin increased the time course and amplitude of compound action potentials in A-fibers of rat sural and vagal nerves, indicating that Na_V (with possible effects on K_V channels as well) were modulated by this agent.[87] The Na_V family consists of nine members; six members are sensitive to the organic blocker tetrodotoxin (TTX) ($Na_V1.1$–1.4, 1.6, and 1.7), and three are insensitive ($Na_V1.5$, 1.8, and 1.9).[88] Subsequent research turned to studying this modulation in heterologous expression systems in order to obtain greater mechanistic insights into chemotherapeutic modulation of Na_V channel function.

At the biophysical level, oxaliplatin can decrease the peak amplitude of Na^+ currents in NG108-15 neuronal cells (and in HEK293 cells in which $Na_V1.5$ channels were heterologously expressed).[66] Oxalate, but not dichlorodiaminocyclohexane platinum (which are the two main metabolites of oxaliplatin) decreases total Na^+ currents in a Ca^{2+}-dependent manner, suggesting that Na^+ current inhibition by oxaliplatin results from Ca^{2+} immobilization by oxalate.[89] In contrast, other groups have shown that oxaliplatin can shift the voltage of activation to more negative potentials as well as slow inactivation at negative membrane potentials, thereby leading to an increase in total Na^+ current[56,87,90] and an increase in membrane excitability.[91] This oxaliplatin-induced stabilization of the open state occurs with $Na_V1.6$ channels.[56]

Animal studies

In vivo models of CIPN have added to our understanding of the important role that Na_V channels play in the pathogenesis of CIPN. In general, TTX blocks the development of paclitaxel-induced mechanical and cold allodynia[69] and blocks oxaliplatin-induced nerve hyperexcitability in mice[90]; those data emphasize that TTX-sensitive Na_V

Table 2. In vivo ion channel modulation in CIPN.

Antineoplastic agent	In vivo ion channel modulation in CIPN
Taxanes	
Paclitaxel	**Na^+ channels**
	• TTX blocks mechanical and cold allodynia[69]
	Ca^{2+} channels
	• N-type inhibitor reduced acute mechanical hyperalgesia and chronic pain[70]
	• T-type inhibitor or $Ca_V3.2^{-/-}$ reversed mechanical and/or cold allodynia/hyperalgesia[54,71,72]
	• $\alpha_2\delta$ inhibitor reduced mechanical allodynia and hyperalgesia[49–52,73]
	TRP channels
	• $TRPA1^{-/-}$ or inhibitor inhibits mechanical and cold allodynia and heat hyperalgesia[74,75]
	• TRPV4 inhibitor inhibits mechanical allodynia and heat hyperalgesia[74,75]
	• TRPV1 antagonists prevent thermal hyperalgesia and mechanical hypersensitivity[53,75]
Platinum compounds	
Cisplatin	**Ca^{2+} channels**
	• N-type inhibitor prevented development of neuropathic pain[55]
	TRP channels
	• TRPA1 antagonist reversed mechanical allodynia[76]
Oxaliplatin	
	Na^+ channels
	• $Na_V1.4$ and $Na_V1.8$ human polymorphisms increase incidence/severity[61]
	• $Na_V1.7$ inhibitor produced anti-hyperalgesia[63]
	• $Na_V1.3^{-/-}$, $Na_V1.7^{-/-}$, $Na_V1.8^{-/-}$, or $Na_V1.9^{-/-}$ mice still experienced mechanical and cold allodynia[77]
	• $Na_V1.9^{-/-}$ alleviates cold hyperalgesia and allodynia[65]
	K^+ channels
	• HCN1 inhibitor prevented cold hypersensitivity and mechanical hyperalgesia[62,78]
	• $TREK1^{-/-}$ and $TRAAK^{-/-}$ mice did not experience cold hypersensitivity[64]
	• $TREK2^{-/-}$ mice did not experience cool hypersensitivity[64]
	• $K_{Ca}2.3$ length of CAG repeats related to neuropathy[79]
	• KCNQ1 inhibitor induced/activator decreased orofacial cold hyperalgesia[67]
	Ca^{2+} channels
	• $\alpha_2\delta$ inhibitor reduced mechanical allodynia and hyperalgesia[50]
	TRP channels
	• TRPM8 inhibitors prevented cold allodynia[60]
	• $TRPA1^{-/-}$ mice or inhibitors blocked/reversed cold and mechanical hyperalgesia[76,80]
Vinca alkaloids	
Vincristine	**Na^+ channels**
	• $Na_V1.8$ anti-sense still experienced mechanical allodynia[81]
	Ca^{2+} channels
	• T-type inhibitor reversed mechanical allodynia/hyperalgesia[72]
	• $\alpha_2\delta$ inhibitor reduced mechanical allodynia/hyperalgesia[52,73]

TRP: transient receptor potential.

channels play a key role in the development of CIPN. Underscoring the role of $Na_V1.6$ in particular is the observation that oxaliplatin-induced increases in TTX-sensitive Na^+ currents were absent in DRGs from $Na_V1.6^{-/-}$ mice.[56] The centrality of $Na_V1.6$ in this process is highlighted by additional gene deletion studies demonstrating that oxaliplatin- or vincristine-induced mechanical or cold allodynia is preserved in $Na_V1.3$, 1.7, 1.8, or 1.9 null mice.[77,81] To exclude a role for these channels in CIPN may, however, be premature. Disease states that alter the expression and function of ion channels are not the same and may not produce the same physiological effect as

deleting the channels *before* the actual disease or event occurs.[47] In support of this idea, genetic analysis of nociceptor ion channel expression in mice exposed to oxaliplatin detected an increase in $Na_V1.8$ mRNA,[62] while $Na_V1.7$ was increased following paclitaxel administration.[48] More compellingly, a tocainide-derived $Na_V1.7$-selective blocker, NeP1, produced anti-hyperalgesia after oxaliplatin administration.[63] The real test for in vivo target engagement is, of course, to test the efficacy of NeP1 in $Na_V1.7$ null mice following the induction of CIPN with oxaliplatin; those studies remain to be done.

A major complication with CIPN is cold hypersensitivity and cold-induced pain. In particular, the acute neuropathy caused by oxaliplatin is worsened by exposure to cold, and one study investigated the role oxaliplatin and its metabolites oxalate and dichlorodiaminocyclohexane platinum in both the acute (cold hyperalgesia/allodynia) versus chronic neuropathy (mechanical allodynia).[92] Sakurai et al.[92] found that oxalate, which can directly modulate Na_V channels, contributes to the cold hyperalgesia/allodynia but dichlorodiaminocyclohexane platinum, which can bind DNA, contributes to mechanical allodynia but not cold-induced neuropathy suggesting a role for Na_V channels in cold hyperalgesia/allodynia. In support of these findings, Na_V channels have been shown to be modulated by cold and identified as cold sensors in nociceptive neurons.[65,93–95] TTX-sensitive channels, but not those that are TTX-insensitive, are inhibited by cooling temperatures, thus TTX-insensitive channels are still active at cooler temperatures and are responsible for mediating cold sensation and pain.[94] Two TTX-resistant Na_V channels have been specifically attributed to sensing cold temperatures. Reducing temperatures shifts the voltage dependence of activation of $Na_V1.8$ to more negative potentials, thereby facilitating its activation and increasing neuronal excitability.[93] In addition, $Na_V1.8$ null mice showed no response to noxious cold and mechanical stimuli at low temperatures.[95] Like $Na_V1.8$, $Na_V1.9$ also shows increased activity in response to cool temperatures; thus, $Na_V1.9$ null neurons showed impaired firing in response to cold temperatures, and $Na_V1.9$ null mice and rats with $Na_V1.9$ knocked down demonstrated increased cold pain thresholds. In fact, disrupting $Na_V1.9$ alleviates oxaliplatin-induced cold hypersensitivity.[65] Interestingly, $Na_V1.9$ acts like an ''amplifier'' in cold sensing nociceptors by opening and producing a large sustained current near the resting potential, thereby amplifying sub-threshold depolarizations in the membrane potential produced by other co-localized ion channels, such as $Na_V1.8$, and increasing overall excitability.[65] In summary, the animal data strongly suggest that $Na_V1.6$ to 1.9 all contribute to CIPN in vivo.

Human studies

In addition to the in vitro and animal studies, there are human studies that further support the idea that Na_V channels contribute to the development of CIPN.[36,61,96] Two independent studies investigated excitability and nerve conduction in patients who had received treatment with oxaliplatin.[36,96] Knowing that Na_V channels play an important role activating and shaping the AP, as well as establishing and maintaining the resting membrane potential, Krishnan et al.[96] used nerve conduction, specifically the compound AP measurements of the refractoriness and relative refractory period duration, to provide a connection between CIPN and Na_V channel dysfunction. The relative refractory period is the time between APs when it is very difficult to generate another AP, and refractoriness is the percentage increase in current that is needed to generate an AP during the relative refractory period. Their results showed that oxaliplatin lead to increases in the refractoriness and relative refractory period, while patients were undergoing treatment and interestingly, they preceded the onset of clinical pain symptoms.[96] These increases were the result of acute administration of oxaliplatin, and it is known that oxaliplatin produces an acute pain syndrome that is caused by the oxaliplatin metabolite, oxalate, directly affecting Na_V channels.[89] They also found that these early changes in the length of the median nerve AP refractory period were a predictor of the development of chronic neuropathy after oxaliplatin treatment, such that 78% of patients that had a refractory period of at least 4 ms (where normal duration is 3 ms), developed peripheral neuropathy,[96] suggesting early changes in Na_V channels could lead to long-term CIPN. In a similar study, Park et al.[36] found that abnormalities in nerve excitability, specifically an increase in what they term ''superexcitability,'' or the period of time after the refractory period when it is much easier to generate an AP, of at least 15% that occurred early in treatment (prior to cycle 5) predicted the clinical outcome of moderate to severe paresthesia in 80% of patients. Collectively, these data indicate early or acute changes to Na_V channels contribute to long-term CIPN in humans and may therefore represent relevant therapeutic molecular targets. But humans are not mice, so the question really becomes *which* human Na_V channels are relevant?

To address this question, Argyriou et al.[61] conducted a prospective multicenter study to identify single nucleotide polymorphisms in Na_V genes that could confer susceptibility to oxaliplatin-induced peripheral neuropathy. They found two polymorphisms, one in $Na_V1.4$, which is associated with increased incidence and severity of peripheral neuropathy, and the other in $Na_V1.8$, which is associated with increased incidence. That $Na_V1.8$ appears here is entirely congruent with the animal literature, while a role for $Na_V1.4$ may be species specific.

Potassium channels

Biophysics

While Na_V channels are widely considered to be key to CIPN, K^+ channels are controversial even though they contribute to the hyperexcitability observed in other pain syndromes.[97] In support, oxaliplatin application broadened the repolarization phase, and the AHP, and increased repetitive firing in rat peripheral myelinated nerve fibers, indicating that oxaliplatin-modulation of K^+ channel inactivation was involved as channel inactivation shapes these electrical properties.[98] Further supporting a role of K_V channels in CIPN, Benoit et al.[91] found that oxaliplatin blocked K^+ channels and shifted the voltage dependence of activation to more negative potentials (although it is three times less effective at blocking K^+ than Na^+ currents). Additionally, oxaliplatin can inhibit delayed rectifier K^+ current amplitude but did not affect their activation and inactivation in NG-108-15 cells.[66] In opposition, however, Adelsberger et al.[87] found that oxaliplatin did not affect K^+ currents in rat nerve preparations, while other studies observed that application of K^+ channel blockers (4-AP, tetraethylammonium, and apamin) did not replicate the effects of oxaliplatin on nerve hyperexcitability and could still block K^+ currents after administration of oxaliplatin.[90] The biophysical data are equivocal with respect to supporting (or not) a role for K_V channels in CIPN.

Animal studies

Despite the somewhat ambiguous biophysical results, data from animal models support a role for K^+ channels in CIPN. The discordant results may arise from the fact that K^+ channels are among a highly diverse class of ion channels, and in neurons alone, there are at least five different subfamilies: voltage-gated (K_V), Ca^{2+} activated (K_{Ca}), inwardly rectifying (K_{ir}), two-pore (K_{2P}), and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.[99] In rats given paclitaxel, contrasting changes in expression of a variety of K^+ channel genes from multiple families were observed; specifically, there were increases in mRNA expression coding for $K_V1.2$, $K_V11.3$, $K_{ir}3.1$, and HCN1 channels along with reductions in $K_{ir}1.1$, $K_{ir}3.4$, and $K_{2P}1.1$ mRNAs.[48] Notably, these changes were accompanied by a significant increase in excitability (as measured by AP firing and rheobase) in medium and large but not small, diameter nociceptors. Oxaliplatin also induced changes in K^+ channel mRNA in mouse DRGs, with a down regulation of TREK1, TREK2, TRAAK, and $K_v1.1$ and an increase in HCN1.[62,64]

It is interesting that an increase in HCN1 mRNA appears to be a common feature across different classes of antineoplastics, suggesting that HCN1 channel regulation may represent a convergent pathway. HCN channels (HCN1-4) are activated by membrane hyperpolarization, are weakly selective for K^+ over Na^+, and are the basis for the pacemaker current, I_h, that contributes to the resting membrane potential and spontaneous firing in neurons.[100–103] Mice treated with the non-isoform selective HCN channel blocker, ivabradine, did not experience oxaliplatin-mediated cold hypersensitivity and mechanical hyperalgesia (Figure 1).[62,78] Given the mRNA results demonstrating an apparent bias for HCN1, it would be worth testing an HCN1-selective channel blocker[104–107] to determine the degree to which a single HCN channel isoform drives CIPN pathophysiology and whether highly restricted targeting represents a viable therapeutic strategy; proof of in vivo target engagement could make use of appropriate gene deletion mouse models.

Other K^+ channels have also been implicated in CIPN. In rats, GIRK1/$K_{ir}3.1$ channels, which conduct an inward K^+ current, are voltage independent, help maintain the resting membrane potential,[108] and are involved in the morphine-induced relief of oxaliplatin-associated neuropathy.[57] Interestingly, in a rat model of orofacial cold hyperalgesia induced by oxaliplatin, KCNQ ($K_V7.1$–7.5) channels (which help regulate neuronal excitability by contributing to the resting membrane potential[109,110]) are key mediators of this hyperalgesia. When the KCNQ blocker linopirdine[109] was injected into the orofacial region, it produced cold hyperalgesia (as demonstrated by reductions in total contact time with a cooling module); however, when the KCNQ activator retigabine[109] was given to rats treated with oxaliplatin, there was a reduction in cold hyperalgesia.[67] Finally, retigabine also partially restored nerve conduction properties and axon loss found in mice treated with cisplatin, indicating it could prevent CIPN.[68] Although these data strongly implicate K_V7 channels as contributing to CIPN, such an interpretation is based on the assumption that the in vivo pharmacology is unambiguous (which is often not as "clean" as one would like) and should be correlated with corresponding changes in mRNA and/or protein levels in order to make a more compelling argument in support of such a role.

K_{2P} potassium "leak" channels, yet another class of K^+ channels involved in transducing noxious (i.e., painful) stimuli, conduct an outward K^+ current that serves to hyperpolarize the resting membrane potential and help regulate neuronal excitability.[111] TREK1, TREK2, and TRAAK are members of the K_{2P} family and are thermosensitive channels that appear to play an important role in mediating cold hypersensitivity.[64,93] TREK1 and TRAAK sense noxious cold temperatures, whereas TREK2 is involved in non-painful moderate cold sensation.[64] As noted above, cold intolerance is a common feature in patients with CIPN. When TREK2 knock-out

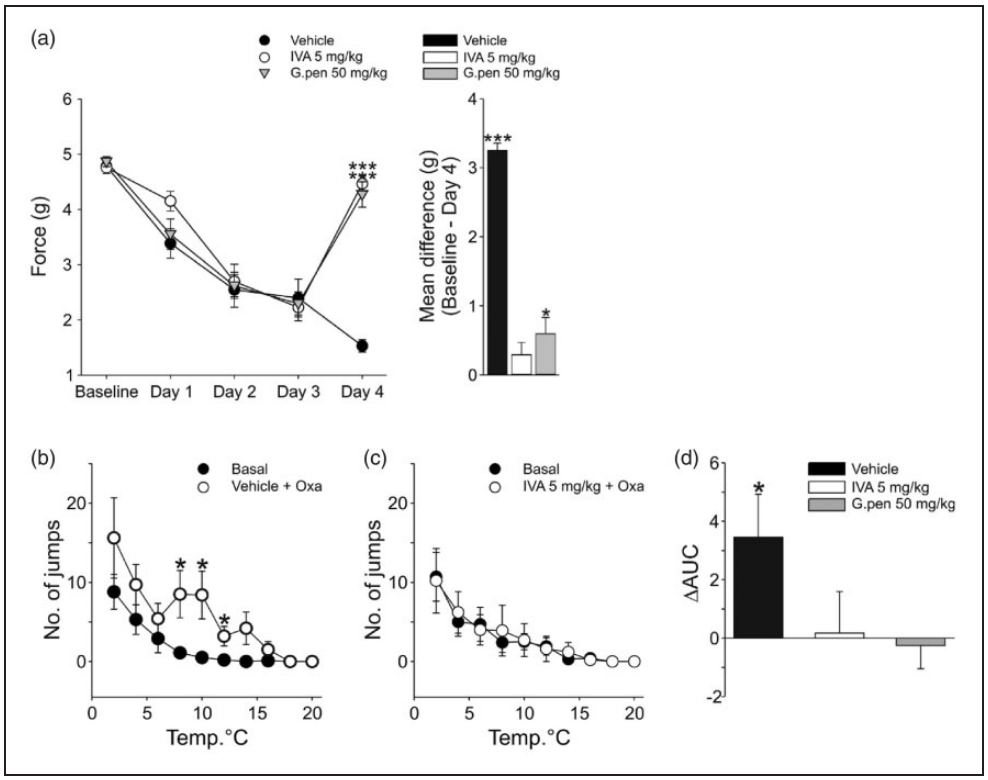

Figure 1. Oxaliplatin-induced mechanical and cold hypersensitivity is reversed by ivabradine. (a) (*Left*) A single dose of oxaliplatin (6 mg/kg) causes a steady decrease in mechanical threshold over four days. On the fourth day, intraperitoneal administration of ivabradine (IVA; 5 mg/kg) or gabapentin (G.pen; 50 mg/kg) causes a significant increase in mechanical threshold when compared to vehicle-treated mice. (*Right*) Mean difference in mechanical threshold on Day 4 compared to pre-oxaliplatin levels for ivabradine, gabapentin, and vehicle-treated animals. Only ivabradine (open bar) returns the mechanical threshold fully to baseline levels. $N = 10$ for each group. (b) and (c) Number of jumps made by mice in response to a cold ramp (cooling from 20°C to 0°C at 2°C/min) before (basal) and four days after a single dose of oxaliplatin (Oxa; 6 mg/kg) with pre-administration (30 min) of vehicle (b) or ivabradine (c; 10 mg/kg). (d) Only the vehicle-treated group shows a significant difference in the number of jumps pre-post oxaliplatin (ΔAUC, difference in total number of jumps in response to temperature ramp), $N = 10$ for each group. Figure modified from Young et al.[78] with permission.

mice were treated with oxaliplatin, they did not display chemotherapy-induced *cool* (i.e., at 20°C–25°C) hypersensitivity; in addition, wild-type mice treated with oxaliplatin, TREK2 mRNA was reduced by almost half in lumbar DRGs.[64] Double (TREK1-TRAAK) and triple (TREK1-TREK2-TRAAK) knock-out mice did not display oxaliplatin-mediated cold (i.e., at ~15°C) hypersensitivity[62,64] emphasizing the roles of TREK1 and TRAAK channels involvement in CIPN at cold temperatures, as opposed to TREK2 involvement in CIPN at cool temperatures.

Another temperature-sensitive class of K^+ channels that may contribute to cold intolerance in CIPN belongs to the "A-type" K^+ channel family. Several different channel proteins (including $K_V1.4$, $K_V3.4$, $K_V4.1$–4.3) conduct a rapidly activating and inactivating A-type K^+ current (I_A) which helps regulate resting membrane potential, current threshold for AP generation, and AP repolarization.[112] I_A currents were significantly reduced at 24°C (by 32%), severely reduced at 15°C (by 74%), and almost eliminated at 10°C (by 88%).[94] The inhibition of I_A can cause hyperexcitability of neurons leading to cold hypersensitivity because I_A essentially provides a brake for excitability; therefore, reduction of this current at cold temperatures releases that brake leading to membrane depolarization, which could then activate other channels.[94] Whether A-type K^+ channels contribute to CIPN is unknown; if they do, however, the participation of $K_V4.2$ is questionable as its expression in DRG neurons is negligible.[113]

Human studies

One human study investigated the role of the small conductance Ca^{2+}-dependent potassium channel, SK3 ($K_{Ca}2.3$), in oxaliplatin-induced neurotoxicity.[79] $K_{Ca}2.3$ is responsible for the AP AHP, thereby helping control neuronal excitability, and is located both in the spinal cord and in DRGs.[114] $K_{Ca}2.3$ channels have a CAG motif that can repeat between 12 and 26 times[115] and

is essential for channel tetramerization. The study by Basso et al.[79] looked at sensory and motor nerve conduction before and after administration of oxaliplatin and found that patients treated with oxaliplatin that had neuropathy also had increased nerve hyperexcitability (manifested as increased amplitude of the compound AP, increased discharge and variable intraburst frequency). Interestingly, this nerve hyperexcitability was related to the length of the CAG repeats found in SK3; specifically, patients with short CAG repeats (13–14) had increased nerve hyperexcitability.[79] These results suggest that the shorter CAG region impairs the assembly or function of SK3 channels resulting in improper AP AHP formation, thereby leading to the increased discharge seen in these studies. In summary, chemotherapeutic agents affect nearly all subfamilies of the K^+ channel superfamily, suggesting that they are key elements in the development of CIPN, which could be considered legitimate molecular targets with respect to the development of new therapeutics.

Calcium channels

Ca^{2+} signaling

While calcium channels (Ca_V) are known to be important contributors to pain signaling,[116] their role in CIPN has not been studied as extensively as that for Na_V and K_V channels. Ca_V can be divided into two groups, high voltage activated and low voltage activated based on the voltage at which they open.[117] The Ca_V1 (L-type) and Ca_V2 (P/Q-, N-, and R-type) channels are members of the high-voltage activated group, and Ca_V3 (T-type) channels are the sole members of the low-voltage activated group.[118,119] In addition to the pore forming α subunit, Ca_V channels also consist of the β and $\alpha_2\delta$ auxiliary subunits, which can enhance trafficking and channel expression as well as affect biophysical properties such as activation and inactivation.[117] Initial studies into the role of Ca_V channels in CIPN focused on changes in intracellular Ca^{2+} concentration, including influx through Ca_V channels themselves as well as Ca^{2+} efflux from mitochondria and other intracellular Ca^{2+} stores.[120] In general, intracellular Ca^{2+} regulation was shown to be disrupted after exposure to chemotherapy agents such as paclitaxel, vincristine, and oxaliplatin.[15,120,121] Additionally, administration of intracellular Ca^{2+} chelators such as EGTA, TMB-8, and Quin-2 inhibited paclitaxel- and vincristine-induced mechano-allodynia and hyperalgesia in rats.[120] Since chemotherapeutic agents affected intracellular Ca^{2+} to cause neuropathy, the next step was to determine if specific plasma membrane Ca_V channels were involved.

Early research investigated global calcium entry via non-specific Ca_V channels on the cell membrane.[122] Cisplatin was shown to reduce peak and sustained Ca^{2+} currents in small diameter DRG neurons[122]; in contrast, however, paclitaxel increased voltage-gated Ca^{2+} currents in small and medium diameter rat DRG neurons.[49] These findings are important because small and medium diameter DRG neurons are responsible for conveying noxious sensory stimuli, suggesting these channels are important mediators of specific sensory abnormalities associated with CIPN. Recent studies have focused on the role of specific Ca_V channels in the pathogenesis of CIPN. Short-term exposure of rat DRG to cisplatin resulted in a decrease in P/Q (i.e., $Ca_V2.1$), L- and T-type (i.e., $Ca_V3.1$–3.3), and Ca^{2+} currents but an increase in the N-type currents.[55] N-type currents and protein levels are also increased after long-term exposure (24–48 h) to cisplatin.[55] Interestingly, there are differences as to how N-type currents are upregulated at these two time points. Short-term increase in N-type currents occurs via protein kinase C (PKC), but long-term changes are mediated via CaMKII.[55] Regulation of intracellular Ca^{2+} has important consequences on cell survival as nimodipine, an L-type (i.e., $Ca_V1.1$–1.4) Ca^{2+} channel inhibitor, or ω-conotoxin, an N-type (i.e., $Ca_V2.2$) Ca^{2+} channel inhibitor, reduce cisplatin-induced DRG neuronal death in vitro.[55,123]

Animal models

In animal models of CIPN, cisplatin administration increased protein, but not mRNA levels of the N-type Ca_V channel, indicating post-translational modifications were responsible for the observed effects.[55] Further supporting evidence for a role for N-type Ca_V channels in CIPN can be found in studies with ω-conotoxin, an N-type Ca_V blocker that is clinically available as ziconotide (Prialt®, Jazz Pharmaceuticals, Dublin, Ireland) and used in the treatment of severe chronic pain. When ω-conotoxin is administered before cisplatin treatment, it prevented upregulation in N-type Ca_V channel expression and the development of neuropathic pain[55]; when administered after paclitaxel treatment, it reduced acute mechanical hyperalgesia and prevented the worsening of chronic pain.[70] Likewise, Pha1b, a peptide toxin that blocks N-type Ca_V channels (similar to ω-conotoxin but with fewer side effects), can reduce paclitaxel-induced acute mechanical hyperalgesia and prevent the exacerbation of chronic pain in rats.[70] T-type Ca_V channels also play an important role in CIPN. When the T-type $Ca_V3.2$ channel is knocked-down or pharmacologically blocked in a rat model of paclitaxel-induced peripheral neuropathy, antineoplastic-induced hyperalgesia is reversed.[71] Similarly, if the anti-epileptic T-type Ca_V channel blocker ethosuximide is administered to rats after paclitaxel (or vincristine) treatment, it can reverse the induced mechanical allodynia, hyperalgesia, and cold

allodynia.[72] Collectively, these results strongly suggest that changes in $Ca_V2.2$ and $Ca_V3.2$ expression or function contribute to CIPN.

A recent elegant study by Li et al.[54] further suggests the importance of $Ca_V3.2$ channels in CIPN. Paclitaxel increased $Ca_V3.2$ channel expression in small DRG neurons that were positive for calcitonin gene-related peptide and isolectin B4, anatomical markers of nociceptive neurons.[124] Following paclitaxel administration, acutely dissociated small diameter (<30 μm) DRG neurons were hyperexcitable (as measured by both spontaneous and depolarization-induced AP firing) compared to neurons obtained from vehicle-treated animals. These small diameter neurons also showed an increase in the T-type calcium current (as well as a shift in the activation curve to more negative potentials and a slowing of inactivation), contributing to increased Ca^{2+} entry.[54] In vivo, paclitaxel-induced mechanical hypersensitivity was prevented by administration of the $Ca_V3.2$-specific inhibitor ML218 when administered to rats before and immediately after paclitaxel treatment, thereby providing additional support for a role of $Ca_V3.2$ in CIPN.[54] Previously, Li et al.[125] found that the toll-like receptor 4 (TLR4) contributed to paclitaxel-induced CIPN; consequently, they investigated its potential role in the increase in $Ca_V3.2$ expression and function. In the present study, the authors demonstrated that $Ca_V3.2$ and TLR4 co-localized to nociceptive neurons and can interact (as shown by co-immunoprecipitation). When a TLR4 inhibitor (TAK242) was administered to rats before and immediately after paclitaxel treatment, the results were similar to those obtained with ML218 described above, with a prevention of mechanical hypersensitivity. Collectively, these results suggest that paclitaxel acts via the TLR4 to upregulate $Ca_V3.2$ expression and function, which in turn results in spontaneous activity of DRG and neuropathic pain.[54] Finally, they were able to replicate the in vitro results using freshly obtained (i.e., non-cadaveric) human DRG neurons, thereby providing the necessary translational link which could support pursuing novel treatment strategies.[54] It is worth noting that the paclitaxel-induced hyperexcitability in DRG neurons decreased between post-treatment day 7 and day 14 (but not to baseline), suggesting that the initial hyperexcitability drives the development of CIPN, while the persistent excitability contributes to chronic neuropathic symptoms. If true, such a scenario has important implications with respect to the timing of any therapeutic intervention targeting Ca_V channel-dependent hyperexcitability (and potentially the other ion channels as well), and that such intervention should be initiated prior to the start of chemotherapy and then continue throughout; how long after the end of chemotherapy such treatment should continue would depend on the pharmacokinetic profile(s) of the given antineoplastic regimen.

As mentioned above, Ca_V channels have auxiliary β and $\alpha_2\delta$ subunits that are important in channel expression and function. Interestingly, antineoplastics also alter $\alpha_2\delta$ subunit expression. Oxaliplatin, cisplatin, and paclitaxel increase $\alpha_2\delta$ expression in DRG neurons,[49–51,55] while paclitaxel increases $\alpha_2\delta$ expression in the dorsal horn.[50,52] Gabapentin, a clinically relevant anti-epileptic drug that binds to the Ca_V $\alpha_2\delta$ subunit,[126] has been shown to have anti-hyperalgesic properties in CIPN. Thus, gabapentin has been shown to inhibit the paclitaxel-induced increase in Ca^{2+} currents in rat DRG neurons, and it normalizes the accompanying paclitaxel-induced increase in $\alpha_2\delta$ expression.[52] At the behavioral level, gabapentin reduced paclitaxel-, vincristine- and oxaliplatin-induced mechano-allodynia and hyperalgesia.[49,50–52,73] As the $\alpha_2\delta$ subunit appears to bind with Ca_V1 and Ca_V2, but not Ca_V3, channels,[127,128] these data strengthen the argument that Ca_V1 and Ca_V2 channels contribute to CIPN (but do not, of course, negate the results above implicating Ca_V3 channels as participants in CIPN pathophysiology).

Human studies

A small retrospective study of patients that underwent chemotherapy with oxaliplatin examined the relationship between Ca_V blocker-administration (presumably administered for their antihypertensive properties) and the incidence of CIPN.[129] Ca_V blockers included any one of the following: amlodipine ($n = 12$), nifedipine ($n = 6$), azelnidipine ($n = 2$), diltiazem ($n = 1$), benidipine ($n = 1$), cilnidipine ($n = 1$), nilvadipine ($n = 1$), or amlodipine + nilvadipine ($n = 1$). Cumulative incidence curves were constructed whereby the probability of acute or chronic neuropathy was plotted against the cumulative dose of oxaliplatin, and the curves of the Ca_V blocker group were compared to the control group. The results indicated that patients who took a Ca_V blocker had a reduced incidence of acute, but not chronic, neuropathy.[129] On the surface, these data are encouraging when contemplating strategies to prevent the development of CIPN, but there is significant heterogeneity among the hypertensives used; while the majority of the drugs tested are L-type (Ca_V1) Ca_V channel blockers, notable exceptions are amlodipine (N- and T-type, Ca_V2 and Ca_V3, respectively), benidipine (L-, N-, and T-type), cilnidipine (N-type), and nilvadipine (T- and L-type).[130–135] Thus, drawing any firm conclusion as to which approach, if any, is the most viable is not possible; furthermore, the effect is only temporary. While this may have some value, it is by no means a panacea.

TRP channels

TRP channels are non-selective cation permeable channels that are highly permeable to Ca^{2+}.[136]

These channels are identified and classified into six subfamilies by their sequence homology to the *trp* gene originally found in *Drosophila*, and they included the following: (1) canonical (TRPC), (2) melastatin (TRPM), (3) vanilloid (TRPV), (4) ankyrin (TRPA), (5) mucolipin (TRPML), and (6) polycystin (TRPP).[136–138] Of those six families, only three are associated with pain, TRPV, TRPA, and TRPM channels.[139] Due to their permeability to Ca^{2+}, these channels may also contribute to the Ca^{2+} disruption caused by chemotherapy agents.[15,120,121] Furthermore, nine TRP channels contribute to thermoregulation and are activated by different temperatures, these channels are as follows: TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4, TRPM5, TRPM8, and TRPA1.[136,137,140] Of the nine, only two are activated by cold (<18°C) temperatures, TRPA1 and TRPM8,[136,137] which suggests that they could contribute to CIPN-associated cold hypersensitivity or cold-induced pain. Consequently, TRP channels have become an important research area for uncovering the etiology of CIPN.

Animal studies

A number of studies have investigated the effect of antineoplastic drugs on the gene and protein expression levels of TRP channels. In this context, oxaliplatin was found to increase the mRNA and protein levels of TRPM8 in rat DRGs and plantar skin samples.[58–60] Furthermore, rats injected with oxaliplatin showed a peak increase in TRPM8 mRNA expression after three days which coincided with the peak of cold allodynia,[58] suggesting an important mechanistic link between CIPN and ion channel regulation. Similarly, paclitaxel has also been shown to increase the mRNA and protein levels of TRPV1 in DRGs[53] and the number of TRPV1-positive cells in DRG neurons.[125]

Most of the studies on the role of TRP channels in CIPN utilize transgenic mice and channel agonists and antagonists to investigate the impact of chemotherapy agents. In one study, TRPA1 knock-out mice and the TRPA1 antagonist HC-030031 were used to block the rapid onset of oxaliplatin- and oxalate-induced cold hypersensitivity.[80] In another study, HC-030031 reversed oxaliplatin-induced mechanical and cold hyperalgesia in rats, and TRPA1 knock-out mice lacked oxaliplatin-induced mechanical and cold hyperalgesia and displayed reduced cisplatin-induced mechanical allodynia.[76] A combination of TRPA1 and TRPV4 blockers (HC-030031 and HC-067047, respectively) completely inhibited paclitaxel-induced mechanical allodynia (which was only partially inhibited by each individual blocker). Further, paclitaxel-induced cold allodynia was markedly reduced in response to TRPA1 pharmacologic block or *Trpa1* gene deletion.[74] The TRPM8 blockers ABMP and TC-I 2014 also stopped oxaliplatin-induced cold allodynia[60]; while the TRPV1 antagonists capsazepine and AMG9810 prevented paclitaxel-induced thermal hyperalgesia and mechanical hypersensitivity, respectively.[53,125] Finally, Chen et al.[75] utilized distinct blockers applied after paclitaxel administration to demonstrate a role for TRP channels in mechanical (TRPA1, TRPV4), and cold (TRPA1) allodynia, and heat hyperalgesia (TRPA1, TRPV4, TRPV1). Overall, the data from pre-clinical animal studies provide strong evidence in support of TRP channels contributing to CIPN.

Human studies

There are limited human data linking this class of ion channels to CIPN. In one study, Li et al.[125] used cultured human DRG neurons and demonstrated that paclitaxel activates and sensitizes TRPV1 channel responses.[125] In another clinical study, the TRPM8 activator menthol was used to assess the role of TRPM8 in CIPN.[141] In this study, menthol was applied to the tongues of healthy subjects and cancer patients before and after chemotherapy treatment with oxaliplatin and determined the cold sensation detection threshold, which is the minimum concentration of menthol needed to produce a cold sensation. The investigators found that there was a decrease in the cold sensation detection threshold after the oxaliplatin chemotherapy treatment, demonstrating hypersensitivity to cold and suggesting a mechanistic role for TRPM8 in CIPN.[141] Although there are strong indications from the pre-clinical animal literature that TRP channels contribute to CIPN, additional information is needed from human (or human tissue) studies to warrant pursuing this channel family as a therapeutic target in CIPN. If and when such data become available, TRP channels would clearly represent a novel target in the treatment of non-noxious temperature-dependent pain syndromes, including CIPN.

Second messenger pathways

The molecular mechanisms that underlie chemotherapy-induced changes in ion channel expression and function, and the resulting peripheral neuropathy, are poorly understood. Thus, even though platinum compounds as a class can produce CIPN, oxaliplatin, but neither cisplatin nor carboplatin, alters Na^{+} channel function, and this effect was recapitulated by oxalate but not by dichloro-diaminocyclohexane platinum.[89] Such a mechanistic divergence within drug class suggests that additional pathways may contribute to the development and/or maintenance of CIPN for any given drug class. Indeed, there are indications that these agents may mediate their effects through other cellular signaling pathways including those that involve pro-inflammatory cytokines,

protein kinases, growth factors, and reactive oxygen species (ROS).[15,73,121,142,143]

Importantly, these second messenger pathways can exert their effect by acting on ion channels.[143–147] For example, protein kinases such as adenosine monophosphate-activated protein kinase (AMPK), mitogen-activated protein kinase, protein kinase A (PKA), and PKC have all been shown to be involved in CIPN.[121,143,148] Concomitant administration of the oral hypoglycemic and AMPK activator metformin to mice with antineoplastics prevented development of mechanical hypersensitivity; such occlusion could result from either (1) mitogen-activated protein kinase-dependent changes in trafficking and/or phosphorylation or (2) AMPK-dependent phosphorylation of ion channels.[143] In addition, PKA and PKCε antagonists can reduce paclitaxel (Taxol)-induced hyperalgesia,[148] and it has been shown that these kinases can modulate ion channel function, including HCN and Na_V channels.[144,145] We will further discuss specific pathways and their role in CIPN with regard to ion channels below.

Cytokines

Cytokines can be released by both glia and neurons and are important in pain pathways.[73] Chemotherapeutic agents induce the upregulation of macrophages leading to an elevated expression of pro-inflammatory cytokines, including TNFα, IL-1β, IL-6, and IL-8.[149] TNFα and IL-1β can sensitize A and C fibers and increase AP discharge via increases in the density of Na^+ and Ca^{2+} currents.[149] Further support for a role for TNFα comes from the observations that TNFα mRNA is increased in a mouse model of vincristine-induced CIPN, while inhibition of TNFα activity using an anti-TNFα antibody prevented mechanical allodynia.[150] In addition, TNFα has been shown to decrease Ca^{2+} currents and increase Na^+ currents when directly applied to nociceptive DRG neurons.[151] Finally, activation of TNF receptors increases Na^+ currents in cultured rat DRG neurons.[146]

With regard to chemotherapeutics, paclitaxel can induce expression of IL-1β, TNFα, and CD11b (a marker of immune cells/macrophages) mRNA in rat DRGs.[152] Moreover, blocking IL-1β with a receptor antagonist or adding the anti-inflammatory cytokine IL-10 reversed and/or prevented paclitaxel-induced mechanical allodynia and decreased the expression of IL-1β, TNFα, and CD11b.[152] Elsewhere it was demonstrated that paclitaxel-induced cold hyperalgesia depends on the chemokine (C-C motif) ligand 2 (CCL2) to activate the C-C chemokine receptor type 2 (CCR2), which in turn activates microglia,[153] and activated microglia are principal participants in the development and maintenance of neuropathic pain.[154] The link between microglia activation, cytokine release, and ion channel modulation is demonstrated by the observation that CCL2/CCR2 can activate PKC/NFκB which can, in turn, phosphorylate the $Na_V1.8$ channel leading to increased current density in inflammation-induced neuropathic pain[155]; such results are entirely consistent with a role of cytokine-dependent signaling in CIPN.[156,157]

Another inflammatory cytokine that may also play a role in CIPN is IL-6, which is thought to be an important mediator in a wide variety of pathologic pain states.[158] IL-6 levels were found to increase in macrophages near the sciatic nerve and DRGs of mice after vincristine treatment.[159] The addition of an anti-IL-6 antibody, which prevents binding of IL-6 to its receptor (IL-6R), reduced mechanical allodynia after its development.[159] A similar picture also emerged in IL-6 knock-out mice treated with vincristine which displayed lower expression of mechanical allodynia.[159] Deletion of glycoprotein 130 (GP130), a membrane protein, and IL-6 receptor that mediates the effect of the IL-6-IL-6R complex, led to a reduction in mechanical and thermal hypersensitivity as well as altered excitability and potassium conductance of nociceptors; specifically, there is increased mRNA levels of Kcna4 and A-type K^+ channel current density connecting cytokine signaling with regulating K^+ channel function in nociceptor excitability and pain.[160] Finally, a breast cancer study that compared the expression of IL-6, and the soluble IL-6 receptor (sIL-6R) after chemotherapy found that painful CIPN was associated with elevated levels of IL-6/sIL-6R, and inversely correlated with QoL categories.[161] The occurrence of painful CIPN symptoms and the reduced QoL were linked to the activation of GP130 by the IL-6 and sIL-6R complex[161]; which further demonstrates its contribution to CIPN and potential as a therapeutic target.

TRP channels and second messengers

The channel family that has been best studied with regard to second messenger modulation by antineoplastics are TRP channels, thereby possibly providing an interesting link between chemotherapy agents, second messenger modulation, ion channels, and CIPN. For example, paclitaxel can sensitize TRPV1 responses to capsaicin when TRPV1 is co-expressed with TLR4, a receptor that when activated triggers downstream mechanisms that lead to secretion of pro-inflammatory cytokines[162] in HEK293 cells, DRGs, and spinal neurons.[125] Interestingly, adding a TLR4 agonist can mimic these results, and a TLR4 antagonist prevents the increase in the number of TRPV1-positive neurons induced by paclitaxel.[125] In addition, TLR4 and TRPV1 are co-localized in rat and human DRG neurons.[125] These results suggest that paclitaxel acts via the TLR4 receptor to sensitize TRPV1 leading to neuropathic pain.

TRPA1 channels are activated by oxidative stress and chemotherapeutics. Oxaliplatin can induce oxidative stress which results in the production of ROS[163]; thus, TRPA1 may represent an important connection between oxidative stress, ROS, and CIPN.[15] For example, oxidative stress agents such as 4-hydroxynonenal and hydrogen peroxide act via TRPA1 and can cause cold hyperalgesia, which mimics oxaliplatin-induced cold hyperalgesia.[15,164] In addition, antioxidants such as acetyl-L-carnitine, α-lipoic acid, or vitamin C inhibit oxaliplatin-induced hyperalgesia.[15,165] Similar results were observed with paclitaxel and TRPA1. Paclitaxel increased the production of ROS, which in turn sensitized TRPA1 channels, while ROS scavengers were shown to block paclitaxel-induced mechanical hyperalgesia.[73]

Chen et al.[75] conducted an elegant study piecing together a pathway from paclitaxel to neuropathic pain via the proteinase-activated receptor 2 (PAR2) pathway and TRP channels.[75] It is known that mast cells release tryptase, an activator of PAR2, and a sign of inflammation,[166] and PLC, PKC, and PKA are all downstream mediators of PAR2. PAR2 is expressed on DRG neurons, is co-expressed with TRPV1, TRPV4, and TRPA1 receptors, and can sensitize these channels via the PKA, PKC, and/or PLC pathway.[75,167–169] Using a mouse model of paclitaxel-induced neuropathy, Chen et al.[75] observed increased release of tryptase from mast cells and an antagonist to PAR2 blocked pain behaviors. This study also found that blocking PLC lessened thermal hypersensitivity, whereas PKCε blockers inhibited mechanical allodynia and heat hyperalgesia, and PKA inhibitors reversed mechanical allodynia, heat hyperalgesia, and cold hyperalgesia. Overall, Chen et al.[75] found that mast cells release tryptase in response to paclitaxel which can then activate PAR2 initiating PKA, PKC, and PLC pathways which can then sensitize TRPV1, TRPV4, and TRPA1 leading to CIPN.

An important study linked Na_V, Ca_V, and TRP channels in the pathway that contributes to oxaliplatin-induced cold hyperalgesia.[59] After application of oxaliplatin or oxalate to rat DRGs, Kawashiri et al.[59] observed cold hyperalgesia and a corresponding increase in TRPM8 mRNA, an increase in Ca^{2+} influx, and the translocation of nuclear factor of activated T-cell (NFAT) to the nucleus, all of which were blocked by L-type Ca^{2+} blockers (nifedipine or diltiazem) or the non-specific Na_V channel blocker mexiletine. These results suggest that: (1) oxaliplatin increases Na^+ currents (see above); (2) the increase in Na^+ current enhances local depolarization in the membrane potential, which facilitates opening of co-localized L-type channels; (3) the increase in Ca_V channel opening results in increased Ca^{2+} influx; (4) the increase in intracellular Ca^{2+} activates NFAT, which then translocates to the nucleus; (5) nuclear NFAT upregulates the expression of TRPM8; and finally, (6) an increase in TRPM8 in the DRG results in cold hyperalgesia.

Strategies to minimize CIPN

Conceptually, there are two ways to mitigate the debilitating effects of CIPN, one is to prevent the neuropathy prior to its onset and the other is to treat the neuropathy after it has developed. To date, although animal models of pain have provided crucial mechanistic insights into the molecular mechanisms of CIPN, whether and how these results will translate into human clinical advances is not yet clear. In this context, several case studies, small trials, and retrospective studies have provided evidence suggesting potential therapeutic strategies for CIPN.[170–173] One case study found that the Na_V blocker lacosamide[174] completely relieved neuropathic pain in patients following chemotherapy associated with vincristine and cisplatin.[175] In another study that utilized the anticonvulsant (and Ca_V $\alpha_2\delta$ channel blocker) pregabalin, patients displayed either complete resolution in their neuropathy or a significant reduction from Grade 3 to Grade 1.[176] Furthermore, in a study involving 23 patients, it was demonstrated that oxaliplatin-induced peripheral neuropathy was reduced in 48% of patients when pregabalin is given after the establishment of CIPN.[177,178] While encouraging, absent large, prospective randomized, controlled trials, these and other data "are insufficient to conclude that any of the purported chemoprotective agents... prevent or limit the neurotoxicity of platin drugs among human patients."[179]

There is still controversy about the efficacy of treatment options for CIPN when expanded to full Phase III randomized, double-blinded studies.[170,172,173,180,181] For example, there have been contrasting reports on the impact of Ca^{2+}/Mg^{2+} infusions on treatment efficacy. In an early retrospective, non-randomized study, oxaliplatin patients who were given Ca^{2+}/Mg^{2+} infusions showed lower neuropathic intensity and recovered more rapidly from neuropathic pain.[182] In contrast, in a placebo-controlled double-blinded prospective trial, there were indications that Ca^{2+}/Mg^{2+} decreased the treatment efficacy of oxaliplatin,[183] leading to early termination; however, a further analysis of the results after the fact demonstrated that there actually was no decrease in treatment efficacy.[184] Another concurrent placebo-controlled randomized study was also terminated early because of the previous negative result; however, when the data were analyzed, the investigators observed a decrease in incidence of Grade 2 or greater sensory neuropathy.[185] Despite a few positive outcomes, there are still concerns with the Ca^{2+}/Mg^{2+} treatment strategy[17,171,186] as a large double-blind, randomized study showed no effect on oxaliplatin-induced peripheral neuropathy.[187] Similar to the Ca^{2+}/Mg^{2+} treatment, other ion channel

modulators including carbamazepine, a Na_V blocker, and gabapentin, a Ca_V channel blocker, also showed promise in some trials[173] but not in others.[173,188]

Anti-oxidants as treatments have also demonstrated some initial positive results.[170,173,189] Amifostine, a free radical scavenger, showed some benefits in preliminary trials with small, but significant, reductions in neuropathy[190,191] but were associated with side effects,[190] and other studies showed a lack of effect,[180,192,193] thus its use is not recommended.[186] The anti-oxidant, glutathione, also revealed mixed results. There were small randomized trials that showed some benefit[194–197]; however, a larger placebo-controlled study did not.[198] Another anti-oxidant and modulator of NGF, acetyl-L-carnitine had similar results, positive findings in small preliminary studies,[173,199] which were not replicated in a larger double-blind placebo-controlled study.[200] That early promising results could not be replicated in subsequent larger studies is not uncommon,[201] and the lack of reproducibility highlights the need to conduct large properly designed studies in the first place.[202–204]

One treatment strategy recently made the transition from positive results in a preliminary trial to a large placebo-controlled trial.[205] Omega-3 fatty acids prevented paclitaxel-induced peripheral neuropathy in a small trial that included patients suffering from breast cancer.[206] Similar results were observed in a recent randomized, double-blind, placebo-controlled study on the effects of n-3 polyunsaturated fatty acids on oxaliplatin-induced peripheral neuropathy in patients with colon cancer. This study also showed a reduction in the incidence and severity of the neuropathy,[205] indicating polyunsaturated fatty acids warrant further investigation.

In 2014, the American Society of Clinical Oncology Clinical Practice published guidelines regarding the prevention and management of CIPN (Table 3)[186,207]; they could not recommend a relevant drug option for the prevention of CIPN due to the lack of high-quality and consistent evidence for the distinct interventions.[186] However, duloxetine, a serotonin and norepinephrine dual re-uptake inhibitor, was their only treatment recommendation due to a positive Phase III clinical trial; it was a qualified recommendation, however, as duloxetine is not universally effective in that it was more effective

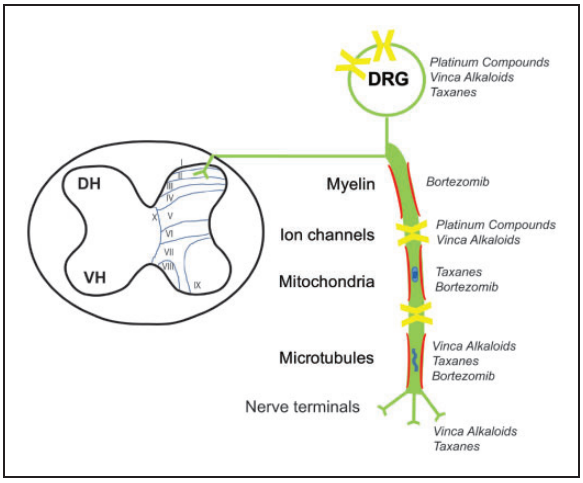

Figure 2. Putative sites of action on peripheral sensory neurons for chemotherapy-induced neuropathy. Major classes of antineoplastics are listed (also shown is bortezomib, a proteasome inhibitor that is used for the treatment of multiple myeloma, which is also associated with CIPN). Primary afferents project to the distinct Laminae of Rexed (schematically illustrated) of the superficial spinal cord dorsal horn (DH) in a topographic fashion; Aδ nociceptors (I), C/Aδ peptidergic fibers (I-II_{outer}), C non-peptidergic fibers (II), Aδ hair follicle afferents (II_{inner}-III), and Aβ hair follicle and tactile afferents (II_{inner}-V).[211] Alterations in neuronal excitability can arise from changes in ion channel function at the level of the cell soma (located in the dorsal root ganglion; DRG), the axon terminal, or along the axon itself.

Table 3. Summary of recommendations from the American Society of Clinical Oncology (ASCO) concerning the prevention of chemotherapy-induced peripheral neuropathy in adult survivors of cancer.

Proposed treatment	Strength of evidence	Recommendation	Benefit(s)	Harm(s)[a]
Acetyl-L -carnitine	Low	Inconclusive	Low	Moderate
Duloxetine	Intermediate	Moderate for	Intermediate	Low
Gabapentin	Intermediate	Inconclusive	Low	Low
Lamotrigine	Intermediate	Moderate against	None demonstrated	Low
Nortriptyline/amitriptyline	Intermediate	Inconclusive	Low	Low
Topical amitriptyline, ketamine, ± Baclofen	Intermediate	Inconclusive	Moderate	Low

Source: Table modified from Hershman et al.[186]

[a]"Harms" were identified by the Clinical Practice Guideline Committee based on the specific clinical trials identified in the review and not on any other evaluations of the safety of those treatments. These recommendations are separate and distinct from the ASCO practice guidelines for the management of chronic pain in survivors of adult cancers.[207]

against oxaliplatin-induced CIPN than that induced by taxanes.[17,186] While none of the current published studies have provided an appropriate therapy, there are currently over 30 clinical trials currently active regarding CIPN treatments which could provide promising results in the future.[208]

So the important question is why is there a lack of high-quality consistent evidence? The lack of progress may be due to the fact that most of the studies that show positive results are generally associated with a small sample size, are un-blinded, or had no placebo controls, and the negative side effects of the agents are generally not reported.[17,170,186] Furthermore, there is also a lack of a universally agreed upon method to assess the occurrence of CIPN in clinical trials. Current CIPN assessment methods include physical exams, patient questionnaires, neuropathy scales and scores, nerve conduction studies, and quantitative sensory testing.[172] In addition, there are no standardized approaches to assess the efficacy of these drugs, with studies reporting a combination of symptom scores, clinical assessments, nerve conduction data, and results from quantitative sensory testing.[172] Therefore, it would be important to create a standardized method for the assessment of CIPN occurrence, as well as assessing the efficacy of the agent tested, in order to identify successful strategies for the prevention and/or treatment of CIPN.[209]

Conclusion/future directions

This review has highlighted data from animal and human studies implicating changes in primary afferent excitability resulting from alterations in ion channel expression and function as a common mechanism leading to the development of CIPN. While other mechanisms have been invoked (Figure 2),[73,156,210] there are abundant data implicating ion channels (Tables 1 and 2) and second messengers such as cytokines in the development of CIPN. This is not an unexpected finding as changes in neuronal excitability appear to be a common feature of other neuropathic pain disorders including painful diabetic neuropathy and those associated with peripheral nerve injury.[47,212,213]

While animal studies have provided many exciting positive results (reviewed by Hama and Takamatsu[214]), the heralded promise of those results has not translated into positive results in human clinical trials. Is it possible that we have been focusing on the wrong targets? Conditional gene deletion (''knockout'') strategies to study the role of various proteins in neuropathic pain have been successfully employed,[215–223] all of which appear to confirm them as reasonable targets. One problem with the gene deletion approach, however, is compensatory up- or down-regulation of other channels or signaling cascades, resulting in cellular and/or synaptic homeostasis.[224–227] As previously noted, another important consideration when interpreting gene deletion studies (at least as they relate to pain) is that the role of a given protein, be it an ion channel or enzyme, in pathological-mediated neuropathic pain may not be equivalent to measuring the initiation/development of such pain when it is constitutively absent.[47] While there are important similarities in changes in ion channel protein expression between rodent and human sensory neurons, there may be just as important differences. These differences may account for the divergent results obtained with in vivo models examining new therapeutics for the treatment of neuropathic pain (see e.g., Jarvis et al.[227] and Ziegler et al.[228]).

The failure to translate the pre-clinical results into effective treatment strategies is multifactorial, with numerous potential solutions, including the development of more realistic pre-clinical models,[47] including the use of a non-human primate (NHP) model.[214,229] In theory, an NHP-model might be an improvement over widely used rodent models, but the use of NHPs has been challenged on ethical and scientific grounds.[230] A viable and rapidly developing approach is to use a model that avoids the use of non-human tissues and which instead incorporates sensory neurons derived from human stem cells.[231,232]

Human stem cells, both embryonic stem cells or induced pluripotent, have been used as models of neurodegenerative disease and have provided promising results.[233] Pluripotent stem cells have also been suggested as an approach for studying ion channelopathies.[234] To date, though, there are few studies using human stem cells as a research paradigm for studying pain-related phenomona.[17,233,236] One such study found human fibroblast-derived nociceptors had functional TRPV1, TRPA1, and TRPM8 channels, generated TTX-resistant action potentials and phasic firing patterns characteristic of nociceptors, and when exposed to oxaliplatin, demonstrated sensitization of TRPV1.[236] Elsewhere, Wheeler et al.[233] successfully modeled CIPN with human-induced pluripotent stem cells. They found when cells were treated with paclitaxel, vincristine, or cisplatin, there were morphological differences in neurites including changes in outgrowth, process length, and outgrowth intensity.[233] The authors also knocked down the TUBB2A gene (whose expression has been shown to be associated with reduced risk of paclitaxel-induced neuropathy) in these cells and observed a reduction in neurite outgrowth after adding paclitaxel, suggesting an increased sensitivity to this chemotherapeutic agent.[233] Overall, these studies indicate that human stem cells could be a realistic pre-clinical *human* model for the study of CIPN.

At the in vivo level, neuroimaging techniques, including magnetic resonance imaging or positron emission tomography, can be used to investigate CIPN.[236–238]

These techniques have been used to study pain across species, from rodent to humans, which makes it a particularly strong tool. Advantages of neuroimaging included the following: (1) the ability to follow the progression of the disease, (2) the ability monitor the network (rather than self-reported) response to various treatment strategies, and (3) utility in *both* pre-clinical and clinical research, thereby allowing direct correlation across in vivo models.[237,239] Neuroimaging can even be informative at the molecular level, with the ability to image receptor distribution as well as ligand: receptor interactions and the corresponding signal transduction pathways.[237] Newer techniques using miniature optical imaging systems that can be attached to the head of awake, freely moving rodents have been developed, and this technology avoids the confounding effects of sedation and restraints routinely employed during in vivo imaging.[211] While these techniques have not been used to study CIPN, they could be an interesting new approach since they have been used successfully to study other pain syndromes.

In summary, CIPN is a pervasive condition that at present has no prevention and only one mildly recommended treatment strategy.[186] Despite the diversity of antineoplastic agents and their therapeutic mechanisms of action, one common theme contributing to the mutual pathophysiology of CIPN is their ability to modulate ion channel expression and function, either directly or through second messengers and inflammatory cytokines. Bridging the gap between the results obtained using the current pre-clinical models with those obtained thus far in humans will be necessary if we are to find effective strategies to treat, or even better, prevent, CIPN.

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