

# Taxane-Induced Neuropathic Pain: Current Evidence and Treating Strategies

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## Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling adverse event of most of commonly used antineoplastic agents. Previous studies have focused on several chemotherapeutic agents and reported that CIPN incidence varies from 19% to >85%. The mechanisms underlying CIPN are currently unknown. However, different theories have been proposed including microtubules dysfunction, mitochondrial dysfunction and mitochondrial toxicity, Glial pathway, substance P pathway, adenosine receptor pathway. CIPN is not simply to treat, and most randomized controlled trials failed to identify an effective therapy. Recent evidence supports the efficacy of serotonin (5-HT) and norepinephrine (NE) dual reuptake inhibitors (SNRI) in the treatment of neuropathy-related pain. Based on current evidence, we can speculate that duloxetine and topical menthol would improve CIPN pain as symptomatic treatment while, based on preclinical data, pifithrin- $\mu$  could be considered in future for the prevention of CIPN.

**Keywords:** Neuropathic cancer pain; Chemotherapy induced peripheral neuropathy; Microtubules

## Background

Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling adverse event of most of commonly used antineoplastic agents. The development of CIPN could lead to dose reduction or chemotherapy withdrawn, increasing cancer-related morbidity and mortality [1,2]. CIPN is a predominantly sensory neuropathy that sometimes can be characterized also by motor and autonomic changes. Taxanes include paclitaxel (Taxol) and docetaxel (Taxotere) that act by inhibiting the disassembly of microtubules by way of binding to the beta-tubulin subunit. As a consequence of taxane inhibition, microtubules become more stable and so dysfunctional, leading to cell death by way of altering the physiological tubule dynamics required for cell division and vital interphase processes. Paclitaxel is used mainly as chemotherapeutic agents for ovarian, breast and non-small cell lung cancer, while docetaxel for breast, non-small cell lung, prostate, gastric, head and neck cancer. Paclitaxel induces a bilateral, distal, symmetrical axonal neuropathy with sensory symptoms (numbness, tingling and burning pain) with in a stocking-and-glove distribution. Moreover a symmetrical loss of sensation carried by both large fibers (proprioception, vibration) and small ones (temperature, pinprick) could be present. Most patients improve or resolve within a 3-6 month period after the discontinuation of treatment, but unfortunately more severe cases remain unsolved. The risk of taxane-induced peripheral neuropathy seems to be dose-related. Some studies have reported less neurotoxicity with weekly paclitaxel dosing in comparison to 3-week dosing when the total dose is kept similar.

## Review

### Epidemiology

Previous authors have studied the association between several chemotherapeutic agents and CIPN and reported a range from 19% to more than 85% [1,2].

Seretny et al. published what we believe to be the first systematic review and metaanalysis of the incidence and prevalence of CIPN [3]. The authors screened 4128 potentially relevant studies, and 138 full texts were examined. 31 studies (involving 4179 patients) met their inclusion criteria. 30 studies reported the incidence of CIPN. 1 study reported CIPN prevalence. 15 were prospective cohort studies, 10 were randomized-controlled trials (RCTs), 5 were nonrandomized controlled trials, and 1 was a cross-sectional cohort study. CIPN prevalence was 68.1% (95% CI=57.7-78.4) within the first month of the end of chemotherapy, 60.0% (36.4-81.6) at 3 months, and 30.0% (6.4-53.5) at 6 months or later. Moreover they reported different studies that showing some single nucleotide polymorphisms (SNPs) associated with CIPN. Those polymorphisms were related to different kind of proteins (voltage-gated sodium channels, Schwann cell function-related proteins, receptors for cell surface collagen, receptors involved in neuronal apoptosis, neuronal crest cell development, and an enzyme involved in pyruvate metabolism). Finally they have examined four studies (701 patients) that reported clinical risk factors for CIPN (baseline neuropathy, a history of smoking, low creatinine clearance, cold allodynia and cold hyperalgesia during chemotherapy treatment). The Pathogenesis of CIPN is currently unknown, now we'll describe a review of different mechanisms that seems to be related to CIPN development.

## Pathogenesis

### Microtubules

Early morphological studies reported neural degeneration when paclitaxel was injected into the sciatic nerve [4-6]. The clinical relevance of these studies is controversial because of the excessive endoneural concentrations of paclitaxel. Recently Flatters et al. have studied the peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy in a rat model, and have reported mitochondrial dysfunction [7]. Adult male Sprague-Dawley rats received paclitaxel, 2 mg/kg/ml or vehicle intraperitoneally (i.p.) on four alternate days. Mechano-allodynia and mechano-hyperalgesia were tested by using three von Frey filaments. They conducted also immunohistochemistry assessment with anti-Activating Transcription Factor 3 (ATF3) antibodies, nerve transverse sections study by electron microscopy with analysis of microtubules and mitochondria. After a short period, paclitaxel group presented a marked and prolonged mechano-allodynia and mechano-hyperalgesia that resolved within 5 months (day 155) after the first injection.

Paclitaxel-induced cold allodynia was significant at day 13 post-initiation of paclitaxel and lasted until day 52. The 725 × magnification observation didn't show any effect on morphology of the saphenous nerve: myelin sheaths structure and blood capillaries were normal, without any of degeneration or occlusion of blood flow. These data were confirmed by quantifying the number of myelinated axons and C-fibers following paclitaxel treatment. Moreover no one difference in ATF3-positive cells at day 11 and day 27 post-treatment were found compared to vehicle-treated nerves. As well as there wasn't difference in the total microtubule density in myelinated axons. In particular Flatters et al. observed swollen and vacuolated mitochondria in C-fibers and myelinated axons after paclitaxel treatment. Unfortunately, these atypical changes that are correlated with paclitaxel-induced mechanical hypersensitivity does not indicate the nature of this dysfunction.

### Mitochondrial dysfunction

Mitochondria are a major cellular source of reactive oxygen species (ROS) like superoxide, hydroxyl radical, nitric oxide, and hydrogen peroxide. Previous studies have demonstrated that ROS was involved in nerve injury-induced and inflammatory pain [8-11] and that systemic administration of a nonspecific ROS scavenger prevented the development of paclitaxel-induced mechanical hypersensitivity and could reverse established paclitaxel-induced mechanical and cold hypersensitivities [8-11]. The protein complexes III and I are located into the inner mitochondrial membrane. They are protein complexes of the electron transport chain and play a crucial role, into the oxidative phosphorylation. As a consequence they are sources of ROS [12,13].

Griffiths et al. have published a study on the effect of rotenone (complex I inhibitor) and Antimycin A (complex III inhibitor) on the development and maintenance of paclitaxel-induced painful peripheral neuropathy [14]. They evaluated, in Sprague Dawley rats, mechanical hypersensitivity with Von Frey filaments and motor coordination by using accelerating Rota-rod and catalepsy ring apparatus. Rats received i.p. injections of 2 mg/kg paclitaxel on four alternate days (days 0, 2, 4, and 6). Rotenone (3 mg/kg or 5 mg/kg) or Antimycin A (0.6 mg/kg) or vehicle were administered i.p. to test if the inhibition of complex I or III could reduce established paclitaxel-induced pain or prevent the development of paclitaxel-induced pain, respectively. They demonstrated that paclitaxel injections determined a marked

mechanical hypersensitivity on days 28/29 after paclitaxel initiation and that rotenone 3 mg/kg significantly attenuated responses to von Frey 15 g at 3 hours after administration. Moreover, one hour after 5 mg/kg rotenone administration, responses to von Frey 8 g and 15 g were significantly inhibited as compared with vehicle-treated group and so they demonstrated that rotenone 5 mg/kg determined a complete reversal of paclitaxel-induced mechanical hypersensitivity but this effect disappeared after 24 h from Rotenone administration. Moreover, they examined the effect of antimycin A and demonstrated that One hour after 0.6 mg/kg Antimycin A administration there was a complete reversal of paclitaxel-induced mechanical hypersensitivity and that this effect also disappeared after 24 h from Antimycin A administration. Finally, Griffiths et al. examined also the effects of prophylactic dosing of rotenone or antimycin A on the development of paclitaxel-induced mechanical hypersensitivity.

The two inhibitor were administered once daily in two kind of way: Once daily for 7 days after paclitaxel treatment; once daily form 1 day before paclitaxel treatment till to the 5<sup>th</sup> day after. They demonstrated that administration 1 or 2 mg/kg rotenone on days 7<sup>th</sup> to 13<sup>th</sup>, didn't show any effect on the development of paclitaxel-induced mechanical hypersensitivity, but only a slight delay in the full development of paclitaxel-induced mechanical hypersensitivity, while the rotenone administration on days -1 to 5 didn't have any effect. Moreover, they examined the effect of antimycin A in this paradigm. They demonstrated that Antimycin A 0.2 or 0.4 mg/kg once daily from 7<sup>th</sup> to 13<sup>th</sup> day, didn't have effect on the development of paclitaxel-induced mechanical hypersensitivity, while antimycin A 0.4 mg/kg once daily form 1 day before paclitaxel treatment till to the 5<sup>th</sup> day after significantly delayed and attenuated the development of paclitaxel-induced mechanical hypersensitivity to all von Freys hairs.

They concluded that, Antimycin A administration before and during paclitaxel therapy significantly attenuated the development of paclitaxel-induced mechanical hypersensitivity and also increase the normal baseline responses to von Frey hairs. It let them to hypnotize a particular effect of Antimycin A administration in naive versus paclitaxel-treated rats. The authors, because of the ability of rotenone in inducing Parkinsonian symptoms, investigated if rotenone and Antimycin A used in their behavioural studies, effected motor function. They demonstrated that 0.6 mg/kg Antimycin A had no one effect on motor function (performance on an accelerating Rota-rod at 1, 3, or 24 h after administration in naive rats). While 5 mg/kg rotenone reduced latencies and altered motor coordination at 3 hours after administration of rotenone.

### Mitotoxicity

The pathophysiology for CIPN could be based on a long-lasting dysfunction in mitochondria of peripheral nerve sensory axons (PNSAs) [15-17]. The peripheral Mitotoxicity hypothesis for CIPN, is based on the concept that mitotoxicity within the PNSAs determinate a persistent energy deficit leading to dysfunction of the sodium-potassium pump and a subsequent increasing in abnormal spontaneous discharge in both A- and C-fibers with degeneration of the sensory afferents' terminal arbors. The triggering mechanisms could be the nitroxidative species Peroxynitrite (PN). Protein nitration by PN could lead to gain or loss of protein function [18,19]. PN formation is also sustained by PN-nitration manganese superoxide dismutase (MnSOD) that leads to its inactivation.

MnSOD which is the most important mitochondrial antioxidant enzyme keeping SO [20]. This mechanism determinate an increased

level of SO/PN in mitochondria with alteration of bioenergetics [21]. Kali Janes in a study published on PAIN in 2013 examined if PN was a mediator of bioenergetic deficits and mitochondrial dysfunction in PNSAs during CIPN [22] and, if a PN scavenger, like MnTE-2-PyP (Mn(III) 5,10,15,20-tetrakis(N-n-hexylpyridinium-2-yl)porphyrin, could be mitoprotective and could prevent CIPN. They conducted behavior tests, such as paw withdrawal for mechano-allodynia and analgesimeter for mechanical hyperalgesia on male Sprague Dawley rat. They also evaluated mitochondrial abnormalities on electron microscopy, ATP assay, immunoprecipitation and measurement of mitochondrial MnSOD activity.

The author showed that oxaliplatin induced mechano-allodynia and mechano-hyperalgesia on day (D) 11 and 12 with peak at D16/17. They demonstrated also that a subcutaneous injections of MnTE-2-PyP5<sup>+</sup> attenuated the development of oxaliplatin-induced neuropathic pain in a dose-dependent manner (0.3-3 mg/kg/d, n=5-7) with ED50s on D25 of 0.6 mg/kg/d (95% Confidence Interval (CI)=0.4-0.8 mg/kg/d) and of 0.4 mg/kg/d (95% CI=0.3-0.5 mg/kg/d) for mechano-allodynia and mechano-hyperalgesia, respectively. No differences were noted for higher dosage. Very relevant is that MnTE-2-PyP5<sup>+</sup> blocked nitration and enzymatic inactivation of MnSOD, reduced the increased prevalence of abnormal mitochondria and attenuated the loss of ATP production. These result stress the concept of the importance of mitochondrial PN-mediated nitroxidative alteration in PNSAs in the development of neuropathic pain evoked by diverse chemotherapeutic agents.

### A3 Adenosine Receptor

It has been recently reported that highly specific A3 adenosine receptor (A3AR) agonism is a valid therapeutic strategy for CIPN [23]. Adenosine acts by four G protein-coupled receptor subtypes: A1AR and A3AR couple to Gi/Gq and A2AAR and A2BAR to Gs/olf/o [24]. IB-MECA or its 2-chloro analogue, Cl-IB-MECA (selective A3AR agonists), has been demonstrated to block CIPN without anticancer effects [23]. The mechanism of the beneficial effect of A3AR agonism remain unclear but different pathways have been suggested: inhibition of redox-sensitive NFjB, modulation of glycogen synthase kinase (GSK); reduction of TNF-a/IL-1b; increased of the anti-inflammatory IL-10 [25]; stimulation of glial production of neuroprotective substances like CCL2 and inhibition of glial-derived proinflammatory cytokines [26].

Janes and Salvemini examined NADPH cytochrome c reductase activity and cytokines expressed in the spinal cord of rats treated with paclitaxel 2 mg/kg for four consecutive days. The authors observed on D16 the reduction of the activation of NADPH oxidase and thus superoxide production in the spinal cord by prophylactic treatment with IB-MECA (0.1 mg/kg/ d; given i.p. from D0 to D15) and they demonstrated that it was A3AR3 relating by using a selective A3AR antagonist (MRS1523). These data was associated with the attenuation of mechano-allodynia and mechano-hyperalgesia. They also demonstrated that IB-MECA was able to reduce paclitaxel-induced activation of NFkB and MAPKs as well as the overproduction of TNF-a and IL-1b, TNF-a and IL-1b a finally to increase the level of the neuroprotective and anti-inflammatory cytokine IL-10 [27].

### Substance P

Terusame et al. have demonstrated that that paclitaxel treatment increases release of substance P, but not of Calcitonin gene-related peptide (CGRP) in the superficial layers of the spinal dorsal horn and

so may contribute to CIPN [28]. Male Wistar rats weighing 250-300 g were used in the present study. Paclitaxel (2 or 4 mg/kg/ml;) was injected intraperitoneally (i.p.) on four alternate days as previously described. n day 7 or 14 after paclitaxel (2 or 4 mg/kg) treatment the spinal cord was prepared for immunohistochemistry with the substance P Immunohistochemistry Staining Kit and rabbit polyclonal antiserum to CGRP. The specimens were analyzed with fluorescence microscopy at 400 magnification. The Authors demonstrated that Paclitaxel (2 and 4 mg/kg) treatment induced a significant increase in the substance P in superficial layers of the spinal dorsal horn at day 14 (2 mg/kg: 24.072.4%; 4 mg/kg: 43.674.6%, P=0.01, n=5) compared with substance P protein expression in cremophor vehicle-treated rats, while CGRP expression did not increase.

### Therapy

CIPN is difficult to treat, and most randomized controlled trials have examined different drugs with a variety of mechanism of action, without finding a valid therapy [29]. Evidence is accumulating which suggest that the characteristics of pain may be the most important factor in predicting a treatment response rather than the underlying aetiology [30,31]. There is a lot of evidence that serotonin (5-HT) and norepinephrine (NE) dual reuptake inhibitors (SNRI) are effective in treating neuropathy-related pain, because 5-HT and NE are key neurotransmitters that are able to inhibit pain transmission. While noradrenergic pathways have an inhibitor effect on the transmission of pain, which also continues in case of neuropathic or chronic pain, serotonin may have also a facilitatory effect particularly in the advanced stages of chronicity ad it is, therefore, pronociceptive [32]. Based on that background Smith et al. conducted a randomized phase III trial on Duloxetine [33].

Inclusion criteria were: patients were  $\geq 25$  years of age; more than Grade 1 sensory CIPN based on the NCI Common Toxicity Criteria for Adverse Events (CTCAE) and reported  $\geq 4/10$  average CIPN-related neuropathic pain  $\geq$  three months beyond chemotherapy completion. Exclusion criteria: neuropathy from any type of nerve compression (e.g. carpal/tarsal tunnel syndrome, radiculopathy, spinal stenosis, brachial plexopathy); leptomeningeal carcinomatosis; severe depression; suicidal ideation; bipolar disease; alcohol abuse; a major eating disorder, and markedly abnormal renal or liver function tests. Patients were 1:1 randomized 1:1 in two groups (Group A or Group B). Group A was treated with duloxetine (60 mg) as initial treatment and placebo as crossover treatment. Group B was treated with placebo as initial treatment and duloxetine as crossover treatment. 231 patients were recruited, 115 into Group A and 116 into Group B. Eleven patients (Group A=6; Group B=5) never received treatment, leaving 220 treated patients. Both groups were similar at baseline, except for pain score (mean and standard deviation (SD)): 6.1 (1.7) Group A; 5.6 (1.6) Group B, p=0.02). At the end of the initial treatment period, patients in the duloxetine group reported a larger decrease in average pain (mean change score=1.06; 95% CI: 0.72, 1.40) than those receiving placebo (mean change score=0.34; 95% CI: 0.01, 0.66) (p=0.003). The effect size attributed to duloxetine was moderately large at 0.513. The observed mean difference in the average pain score between the duloxetine and placebo groups was 0.73 (95% CI: 0.26, 1.20).

The authors demonstrated also a greater improvement in CIPN-related Quality Of Life in duloxetine-treated patients compared to the placebo-treated group. During the initial treatment period, the mean change in the FACT/GOG-NTX total score was 2.44 (95% CI: 0.43,

4.45) for duloxetine-treated patients compared to 0.87 (95% CI: 1.09, 2.82) in the placebo group ( $p=0.03$ ). Antidepressants are among the oldest drugs used for the treatment of neuropathic pain. They originally came to be used in the treatment of chronic pain, and in particular neuropathic pain, because some of the patients suffering from chronic pain are also depressed, and these drugs relieve pain as well as depression. However, an independent analgesic action has been reported for TCAs since the 1960s. The relief can be more rapid in some patients and appears to occur at a lower dose than the antidepressant effect. An early concept of the mechanism of antidepressant analgesia was that these drugs are capable of potentiating the activity of the descending inhibitory pathways extending from the brain stem to the dorsal horn of the spinal cord, mainly by inhibiting the reuptake of serotonin and noradrenaline that descending fibers release into the spinal synapses between nociceptors (or first-order neurons) and the spinothalamic neurons (or second-order neurons) [34].

Another symptomatic therapy could be the menthol. There is mounting evidence that that endogenous neural circuitry underlying cooling-induced analgesia may represent a novel therapeutic target [35,36]. Fallon et al. have demonstrated that a topical agent, by the activation of the transient receptor potential melastatin (TRPM) ion channel, have produced significant analgesia [37]. Subsequently he conducted a proof-of-concept study with the objective to demonstrate if 4-6 weeks of treatment with topical 1% menthol in aqueous cream alleviate neuropathic pain. He enrolled Fifty-one patients and used the short-form BPI to assess pain and the Hospital Anxiety and Depression scale (HADS). He also examined functional performance like walking ability (using a GAITRite®), hand dexterity and Quantitative Sensory Testing (QST). 40 of 51 patients completed the treatment. 10 patients dropped out for different reasons. 82% of patients improved in their pain scores. HADS improved too as well as HADS anxiety score and catastrophising. Finally the authors observed an improvement in walking velocity and cadence, while there was no significant improvement in hand dexterity. The authors noted also that the percentage of distal limb skin with abnormal sensation in response to brush, cool and warm stimuli became more distal [38].

Very interesting are the literature data about the prevention of CIPN. Krukowski et al. proposed that prevention of chemotherapy-induced mitochondrial dysfunction may be a promising avenue for inhibition of CIPN [39]. The small-molecule inhibitor pifithrin- $\mu$  (PFT- $\mu$ ), 2-phenyl-ethanesulfonamide, is a specific inhibitor of stress-inducible Hsp70, which induced tumor cell death but markedly showed less toxic to non-transformed cells. Heat shock protein (Hsp) family is a group of conserved molecular chaperons that facilitate proper protein folding, modification, and transportation, and are known as inhibitors of apoptosis [40]. Hsp70 is a member of Hsps, and Hsp70 over-expression has been reported to be associated with a wide range of malignancies [40]. The small-molecule inhibitor pifithrin- $\mu$  (PFT- $\mu$ ) has been identified for its capacity to inhibit mitochondrial p53 accumulation without impacting p53 transcriptional activity. His group have previously demonstrated that the disruption of the p53 mitochondrial pathway and the intraperitoneally administration of PFT- $\mu$  protects against cerebral neuronal loss in a rodent model of neonatal ischemic brain damage. In this model, PFT- $\mu$  prevented mitochondrial accumulation of p53 in the brain, thereby reducing oxidative stress and maintaining ATP production [41].

They hypothesized that the protection of neuronal mitochondria by PFT- $\mu$  might also be a mean to prevent CIPN. Therefore, they also explored possible interference of PFT- $\mu$  with *in vitro* tumor cell killing induced by paclitaxel. To investigate if PFT- $\mu$  administration prevents paclitaxel-induced mechanical allodynia, adult C57/Bl6 mice were treated with 10 mg/kg paclitaxel 6-8 mg/kg PFT- $\mu$ , by intraperitoneally injection, every other day for 2 weeks. In mice treated with paclitaxel alone, decreased paw withdrawal thresholds were observed as measured by the von Frey test for 5 weeks after paclitaxel administration. This decrease in paw-withdrawal threshold is indicative of chemotherapy-induced mechanical allodynia. They demonstrated that Pifithrin- $\mu$  completely prevented the development of paclitaxel-induced decrease in paw withdrawal thresholds. No differences in paw withdrawal threshold were detected between mice treated with vehicle alone, PFT- $\mu$  alone, or PFT- $\mu$  plus paclitaxel. These data demonstrate that systemic administration of PFT- $\mu$  completely prevents paclitaxel-induced mechanical allodynia.

As previously described paclitaxel induces the formation of swollen and vacuolated mitochondria in peripheral sensory neurons that may contribute to CIPN. Using transmission electron microscopy, they confirmed that mitochondria in peripheral sensory nerve cell bodies in lumbar Dorsal Root Ganglia (DRGs) and axons in the sciatic nerve of paclitaxel-treated mice were enlarged in shape, had disorganized cristae, displayed swelling/vacuolization, and loss of double membranes. Furthermore, the number of atypical mitochondria in DRG and nerves was increased after paclitaxel treatment. They demonstrated that administration of PFT- $\mu$  completely prevented these paclitaxel-induced alterations in mitochondrial morphology in the DRGs and sciatic nerve with similar percentages of atypical mitochondria as the mice were treated with PFT- $\mu$  alone. No differences in mitochondrial size were measured when comparing the DRG and sciatic nerves of mice that received PFT- $\mu$  alone or PFT- $\mu$  plus paclitaxel.

Preliminary analysis of mitochondrial abnormalities did not show differences between vehicle alone-treated or PFT- $\mu$  alone-treated mice; therefore, PFT- $\mu$  alone-treated mice were used as baseline. The percentage of atypical mitochondria in samples from mice treated with PFT- $\mu$  alone was consistent with the percentage reported under baseline conditions in previously published studies. These data demonstrate that PFT- $\mu$  efficiently preserves mitochondrial morphological integrity of peripheral sensory neurons in paclitaxel-treated mice. They analyzed the potential protective effect of PFT- $\mu$  on loss of intraepidermal nerve fiber retraction (IENFs) in the hind paws of paclitaxel-treated mice. The density of nerve fibers entering the epidermis was significantly reduced in the paclitaxel-treated mice when compared with the PFT- $\mu$  plus vehicle-treated mice.

Notably, PFT- $\mu$  administration prevented paclitaxel-induced IENFs (usually associated to disruption of mitochondrial function). No differences in IENF levels were observed when comparing the PFT- $\mu$  and the PFT- $\mu$  plus paclitaxel-treated mice. These data demonstrate that PFT- $\mu$  administration protects completely against paclitaxel-induced IENFs loss. One of the potential risks of treating CIPN with PFT- $\mu$  may be that the drug may also protect against paclitaxel-induced tumor cell death by preventing accumulation of p53 in mitochondria. Therefore, they assessed the effect of PFT- $\mu$  on ovarian tumor cells treated *in vitro* with paclitaxel. Human ovarian (p531) tumor cells (HeyA8) were incubated with paclitaxel alone or in combination with PFT- $\mu$ . Tumor cell survival was measured by an enzymatic assay. Culturing HeyA8 cells with paclitaxel alone led to a

reduction in tumor cell survival of more than 50%. Addition of PFT- $\mu$  to the cultures of tumor cells and paclitaxel further reduced ovarian tumor cell survival in comparison with paclitaxel alone (striped bars).

Additionally, PFT- $\mu$  (20 mM) alone also decreased tumor cell survival (solid bars). These data provide evidence that PFT- $\mu$  does not inhibit but conversely enhances the antitumor effect of paclitaxel. Next, they investigated if PFT- $\mu$  also prevented cisplatin-induced neuropathy. Mice were treated with cisplatin (2.3 mg/kg) alone or in combination with PFT- $\mu$ , and mechanical allodynia was measured. In mice treated with cisplatin alone, decreased paw withdrawal thresholds were measured after cisplatin administration at weeks 3, 5, and 7. Systemic PFT- $\mu$  administration completely prevented cisplatin-induced changes in paw withdrawal threshold and thereby cisplatin-induced mechanical allodynia.

After examination of literature on gabapentin and CIPN prevention we found only anecdotal reports [42]. A pilot study was conducted to obtain data to support or refute the utility of pregabalin for the prevention of P-APS (acute pain syndrome) and CIPN [42,43]. Shinde et al. published a multicentric, randomized, double-blinded, pilot trial. They recruited 46 patients with 1:1 randomization in order to be treated or with Pregabalin 75 mg or placebo twice daily, starting from the first dose to chemotherapy till to the last one. CIPN was measured using the European Organization for Research and Treatment of Cancer Quality of Life (EORTC-QLQ) CIPN20 questionnaire. Growth curve models and AUC analysis, showed no significant differences in the EORTC CIPN20 sensory sub-scale ( $p=0.88$  and  $p=0.46$ , respectively) between arms as well as there were no differences in the motor neuropathy or autonomic neuropathy subscales. They found only a small difference in numbness symptom. So these data are unable to determine if gabapentinoids was effective in established CIPN and to provide support in order to conduct a formal phase III clinical trial.

## Conclusions

All mechanisms exposed have a crucial role in the development of CIPN: Microtubules dysfunction; Mitochondrial dysfunction and mitotoxicity; Substance P; Adenosine Receptor. Based on the different trials analyzed, our hypothesis is that duloxetine and topical menthol would ameliorate CIPN pain, but they represent only a symptomatic treatment while, basing on preclinical data, pifithrin- $\mu$  could represent in future a potential therapeutic strategy for prevention of CIPN.

## References

1. Seretny M, Currie GL, Sena ES, Ramnarine S, Grant R, et al. (2014) Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain* 155: 2461-2470.
2. Cavaletti G, Marmiroli P (2010) Chemotherapy-induced peripheral neurotoxicity. *Nat Rev Neurol* 6: 657-666.
3. Hershman DL, Lacchetti C, Dworkin RH, Lavoie Smith EM, Bleeker J, et al. (2014) Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 32: 1941-1967.
4. Roytta M, Horwitz SB, Raine CS (1984) Taxol-induced neuropathy: Short-term effects of local injection. *J Neurocytol* 13: 685-701.
5. Roytta M, Raine CS (1985) Taxol-induced neuropathy: Further ultrastructural studies of nerve fibre changes in situ. *J Neurocytol* 14: 157-175.
6. Roytta M, Raine CS (1986) Taxol-induced neuropathy: Chronic effects of local injection. *J Neurocytol* 15: 483-496.
7. Flatters SJ, Bennett GJ (2006) Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: Evidence for mitochondrial dysfunction. *Pain* 122: 245-257.
8. Nohl H, Gille L, Kozlov A, Staniek K (2003) Are mitochondria a spontaneous and permanent source of reactive oxygen species? *Redox Rep* 8: 135-141.
9. Lee I, Kim HK, Kim JH, Chung K, Chung JM (2007) The role of reactive oxygen species in capsaicin-induced mechanical hyperalgesia and in the activities of dorsal horn neurons. *Pain* 133: 9-17.
10. Schwartz ES, Kim HY, Wang J, Lee I, Klann E, et al. (2009) Persistent pain is dependent on spinal mitochondrial antioxidant levels. *J Neurosci* 29: 159-168.
11. Schwartz ES, Lee I, Chung K, Chung JM (2008) Oxidative stress in the spinal cord is an important contributor in capsaicin-induced mechanical secondary hyperalgesia in mice. *Pain* 138: 514-524.
12. Turrens JE, Alexandre A, Lehninger AL (1985) Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch Biochem Biophys* 237: 408-414.
13. Turrens JE, Boveris A (1980) Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* 191: 421-427.
14. Griffiths LA, Flatters SJ (2015) Pharmacological modulation of the mitochondrial electron transport chain in paclitaxel-induced painful peripheral neuropathy. *J Pain* 16: 981-994.
15. Xiao WH, Bennett GJ (2012) Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. *Pain* 153: 704-709.
16. Xiao WH, Zheng H, Bennett GJ (2012) Characterization of oxaliplatin-induced chronic painful peripheral neuropathy in the rat and comparison with the neuropathy induced by paclitaxel. *Neuroscience* 203: 194-206.
17. Zheng H, Xiao WH, Bennett GJ (2011) Functional deficits in peripheral nerve mitochondria in rats with paclitaxel and oxaliplatin-evoked painful peripheral neuropathy. *Exp Neurol* 232: 154-161.
18. Ischiropoulos H (2009) Protein tyrosine nitration- An update. *Arch Biochem Biophys* 484: 117-121.
19. Macmillan-Crow LA, Cruthirds DL (2001) Invited review: Manganese superoxide dismutase in disease. *Free Radic Res* 34: 325-336.
20. McCord JM, Fridovich I (1969) Superoxide dismutase- An enzymic function for erythrocyte (hemocypre). *J Biol Chem* 244: 6049-6055.
21. Doyle T, Chen Z, Muscoli C, Bryant L, Esposito E, et al. (2012) Targeting the overproduction of peroxynitrite for the prevention and reversal of paclitaxel-induced neuropathic pain. *J Neurosci* 32: 6149-6160.
22. Janes K, Doyle T, Bryant L, Esposito E, Cuzzocrea S, et al. (2013) Bioenergetic deficits in peripheral nerve sensory axons during chemotherapy-induced neuropathic pain resulting from peroxynitrite-mediated post-translational nitration of mitochondrial superoxide dismutase. *Pain* 154: 2432-2440.
23. Chen Z, Janes K, Chen C, Doyle T, Bryant L, et al. (2012) Controlling murine and rat chronic pain through A3 adenosine receptor activation. *FASEB J* 26: 1855-1865.
24. Fredholm BB, Ijzerman AP, Jacobson KA, Linden J, Muller CE (2011) International Union of Basic and Clinical Pharmacology, LXXXI, Nomenclature and classification of adenosine receptors- An update. *Pharmacol Rev* 63: 1-34.
25. Hasko G, Szabo C, Nemeth ZH, Kvetan V, Pastores SM, et al. (1996) Adenosine receptor agonists differentially regulate IL-10, TNF-alpha, and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J Immunol* 157: 4634-4640.
26. Wittendorp MC, Boddeke HW, Biber K (2004) Adenosine A3 receptor-induced CCL2 synthesis in cultured mouse astrocytes. *Glia* 46: 410-418.
27. Janes K, Esposito E, Doyle T, Cuzzocrea S, Tosh DK, et al. (2014) A3 adenosine receptor agonist prevents the development of paclitaxel-

- induced neuropathic pain by modulating spinal glial-restricted redox-dependent signaling pathways. *Pain* 155: 2560-2567.
28. Chiba T, Oka Y, Kambe T, Koizumi N, Abe K, et al. (2016) Paclitaxel-induced peripheral neuropathy increases substance P release in rat spinal cord. *Eur J Pharmacol* 770: 46-51.
  29. Saarto T, Wiffen PJ (2007) Antidepressants for neuropathic pain. *Cochrane Database Syst Rev* 17: CD005454.
  30. Attal N, Bouhassira D, Baron R, Dostrovsky J, Dworkin RH, et al. (2011) Assessing symptom profiles in neuropathic pain clinical trials: Can it improve outcome? *Eur J Pain* 15:441-443.
  31. Dworkin RH, Turk DC, Katz NP, Rowbotham MC, Peirce-Sandner S, et al. (2011) Evidence-based clinical trial design for chronic pain pharmacotherapy: A blueprint for Action. *Pain* 152: S107-S115.
  32. Aurilio C, Pace MC, Pota V, Sansone P, Barbarisi M, et al. (2009) Opioids switching with transdermal systems in chronic cancer pain. *J Exp Clin Cancer Res* 7: 28-61.
  33. Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, et al. (2013) Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: A randomized clinical trial. *JAMA* 309: 1359-1367.
  34. Fornasari D (2017) Pharmacotherapy for neuropathic pain: A review. *Pain Ther* 6: S25-S33.
  35. Liu Y, Qin N (2011) TRPM8 in health and disease: Cold sensing and beyond. *Adv Exp Med Biol* 704: 185-208.
  36. Eid SR, Cortright DN (2009) Transient receptor potential channels on sensory nerves. *Handb Exp Pharmacol* 194: 261-281.
  37. Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, Anderson H, et al. (2006) Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr Biol* 16: 1591-1605.
  38. Fallon MT, Storey DJ, Krishan A, Weir CJ, Mitchell R, et al. (2015) Cancer treatment-related neuropathic pain: Proof of concept study with menthol-A TRPM8 agonist. *Support Care Cancer* 23: 2769-2777.
  39. Krukowski K, Nijboer CH, Huo X, Kavelaars A, Heijnen CJ (2015) Prevention of chemotherapy-induced peripheral neuropathy by the small-molecule inhibitor pifithrin- $\mu$ . *Pain* 156: 2184-2192.
  40. Yang Z, Jingping Ma (2017) Pifithrin- $\mu$  is efficacious against non-small cell lung cancer via inhibition of heat shock protein 70. *Oncology reports* 37: 313-322.
  41. Matsumoto M, Inoue M, Hald A, Xie W, Ueda H (2006) Inhibition of paclitaxel-induced A-fiber hypersensitization by gabapentin. *J Pharmacol Exp Ther* 318: 735-740.
  42. Clarke H, Bonin RP, Orser BA, Englesakis M, Wijeyesundera DN, et al. (2012) The prevention of chronic postsurgical pain using gabapentin and pregabalin: A combined systematic review and meta-analysis. *Anesth Analg* 115: 428-442.
  43. Shinde SS, Seisler D, Soori G, Atherton PJ, Pachman DR, et al. (2016) Can pregabalin prevent paclitaxel-associated neuropathy? - An ACCRU pilot trial. *Support Care Cancer* 24: 547-553.