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Characterization of oxaliplatin-induced chronic painful peripheral neuropathy in the rat and comparison to the neuropathy induced by paclitaxel

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Abstract

Anti-neoplastic agents in the platinum-complex, taxane, vinca alkaloid, and proteasome inhibitor classes induce a dose-limiting, chronic, distal, symmetrical, sensory peripheral neuropathy that is often accompanied by neuropathic pain. Clinical descriptions suggest that these conditions are very similar but clinical data are insufficient to determine the degree of similarity and to determine if they share common pathophysiological mechanisms. Animal models do not have the limitations of clinical studies and so we have characterized a rat model of chronic painful peripheral neuropathy induced by a platinum-complex agent, oxaliplatin, in order to compare it to a previously characterized model of chronic painful peripheral neuropathy induced by a taxane agent, paclitaxel. The oxaliplatin model evokes mechano-allodynia, mechano-hyperalgesia, and cold-allodynia that have a delayed onset, gradually increasing severity, a distinct delay to peak severity, and duration of about 2.5 months. There is no effect on heat sensitivity. EM analyses found no evidence for axonal degeneration in peripheral nerve and there is no up-regulation of activating transcription factor-3 in the lumbar dorsal root ganglia. There is a statistically significant loss of intraepidermal nerve fibers in the plantar hind paw skin. Oxaliplatin treatment causes a significant increase in the incidence of swollen and vacuolated mitochondria in peripheral nerve axons, but not in their Schwann cells. Nerve conduction studies found significant slowing of sensory axons, but no change in motor axons. Single fiber recordings found an abnormal incidence of A- and C-fibers with irregular, low-frequency spontaneous discharge. Prophylactic dosing with two drugs that are known to protect mitochondria, acetyl-L-carnitine and olesoxime, significantly reduced the development of pain hypersensitivity. Our results are very similar to those obtained previously with paclitaxel and support the hypothesis that these two agents, and perhaps other chemotherapeutics, produce very similar conditions because they have a mitotoxic effect on primary afferent neurons.

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Conflict of interest statement

None of the authors has any conflict of interest with respect to the contents of this report.

Keywords

chemotherapy neuropathy; carnitine; olesoxime; mitotoxicity; sensory neuropathy

1 Introduction

Anti-neoplastic agents in the platinum-complex, taxane, vinca alkaloid, and proteasome-inhibitor classes produce a chronic, bilateral, distal, symmetrical, sensory peripheral neuropathy that is often accompanied by a neuropathic pain condition. The chronic sensory symptoms appear in the feet, or in the feet and hands, and include pain, tingling and numbness. Chronic motor dysfunction with a matching distal and symmetrical distribution is absent or rare. Sensory symptoms appear after cumulative dosing and continue to worsen, or sometimes appear for the first time, between treatment cycles (the “coasting” effect) (Argyriou et al., 2008; Cata et al., 2006; Cersosimo, 2005; Quasthoff and Hartung, 2002; Verstappen et al., 2003). The symptoms may last for months to years (Argyriou et al., 2008; Binder et al., 2007; Pietrangeli et al., 2006). The chronic neuropathy is the most common cause of dose reduction and discontinuation of what is otherwise life-saving therapy and it results in a serious decrease in the quality of life for patients under treatment, patients in remission, and for cancer survivors (Paice, 2010; Quasthoff and Hartung, 2002; Windebank and Grisold, 2008).

The anti-cancer mechanism of action of the platinum-complex agents is due to the formation of platinum adjuncts between adjacent DNA bases. The anti-cancer mechanisms for agents in other classes are distinctly different: taxanes and vinca alkaloids interfere with the dynamics of mitotic spindle assembly, and the proteasome-inhibitors disrupt the processing of nascent proteins. Despite this diversity, clinical reports suggest that the chronic peripheral sensory neuropathies produced by agents from all of these classes are very similar (Cata et al., 2006; Quasthoff and Hartung, 2002; Windebank and Grisold, 2008) and all of these conditions are subsumed under the same generic term, chemotherapy-induced peripheral neuropathy (CIPN). However, it is not known whether the neuropathies are due to the same cause. The data from patients suggest that they may be the same, but such data do not allow a clear conclusion because the clinical situation is so complex. Patients do not always receive identical treatment, they often have multiple potential causes of peripheral neuropathy and pain (prior and/or concurrent treatment with other neurotoxic chemotherapeutics, prior radiation therapy, co-morbidities like diabetes, etc.), and only limited kinds of data can be obtained from patients.

Comparisons of animal models of CIPN have fewer limitations. Case-to-case heterogeneity is minimized and measurements that are difficult or impossible to do in a patient can be made with ease. Here we present the characteristics of a rat model of oxaliplatin-induced chronic painful peripheral neuropathy and compare this with the neuropathy induced by a taxane agent, paclitaxel. The behavioral, morphometric, electrophysiological, and pharmacological effects seen with paclitaxel have been characterized in prior work (Bennett et al., 2011; Flatters and Bennett, 2006; Flatters et al., 2006; Jin et al., 2008; Polomano et al., 2001; Siau et al., 2006; Xiao and Bennett, 2008a; Xiao et al., 2009; Xiao et al., 2011). We have compared all of these effects with those seen in the oxaliplatin model, using the same methods that were used for paclitaxel.

It is important to note that oxaliplatin also produces an acute neuropathy that is not seen with other platinum-complex agents (e.g., carboplatin or cisplatin) or with any other chemotherapeutic agent. The acute neuropathy is due to an effect of the oxalate salt on axonal sodium channels (Adelsbeger et al., 2000; Grolleau et al., 2001; Sakurai et al., 2009;

Webster et al., 2005). The work reported here focuses on oxaliplatin's chronic peripheral neuropathy.

2 Materials and methods

2.1 Ethics

These experiments conformed to the ethics guidelines of the International Association for the Study of Pain (Zimmermann, 1983), the National Institutes of Health (USA), and the Canadian Institutes of Health Research. All experimental protocols were approved by the Animal Care Committee of the Faculty of Medicine, McGill University, in accordance with the regulations of the Canadian Council on Animal Care.

2.2 Strategy

The oxaliplatin treatment protocol was chosen on the basis of pilot studies that indicated that this was the approximate minimal dose required for the reliable production of mechano-allodynia and mechano-hyperalgesia. The same strategy was used in selecting the paclitaxel dosing protocol used in prior work (Polomano et al., 2001).

2.3 Animals

Adult male Sprague-Dawley rats (175–250 g, Harlan Inc., Indianapolis, IN; Frederick, MD breeding colony) were housed on sawdust bedding in plastic cages. Artificial lighting was provided on a fixed 12 hour light-dark cycle with food and water available *ad libitum*.

2.4 Oxaliplatin administration

A stock solution of oxaliplatin (Sanofi-Aventis; 5 mg/ml) was diluted to 2 mg/ml with 5% dextrose in distilled water and injected IP at 2 mg/kg on five consecutive days (D0–D4) for a total dose of 10 mg/kg. Control animals received vehicle injections. Animals were weighed daily during treatment and weekly thereafter.

2.5 Renal function

Platinum is nephrotoxic and kidney damage can lead to uremic polyneuropathy (Krishnan and Kiernan, 2007). To examine whether this occurred with our treatment protocol, vehicle-injected controls and oxaliplatin-treated rats were sampled on D7 (n = 10/group) and D35 (n = 12/group), i.e., 3 and 31 days after the last injection of oxaliplatin. After an overdose of sodium pentobarbital (150 mg/kg, IP), urine was collected via bladder puncture and blood was obtained via cardiac puncture. Blood was collected in heparinized tubes and plasma was obtained via centrifugation. The following were measured: blood urea nitrogen (BUN; Quantichrom Urea Assay Kit; BioAssay Systems; Hayward, CO); creatinine in plasma and urine (Enzymatic Creatinine Test Kit; Diazyme Laboratories; Poway, CA); N-acetyl-β-D-glucosaminidase level in urine (NAG; Diazyme Labs; Poway, CA); and protein level in urine (Bio-Rad Dye Reagent; Bio-Rad Laboratories; Hercules, CA).

2.6 Behavioral testing

Animals were habituated to the behavioral testing environment and two baseline measurements were taken prior to oxaliplatin administration. All subsequent behavioral measures were obtained by an observer who was blind as to group assignment. The methods were the same as those used previously (Flatters and Bennett, 2004, 2006)

2.6.1 Mechano-allodynia and mechano-hyperalgesia—The time courses of mechano-allodynia and mechano-hyperalgesia were assessed with von Frey hairs (VFH). A 4 g VFH was used to assess mechano-allodynia. This stimulus rarely evokes a withdrawal

response in the normal animal and evokes a clearly non-noxious touch sensation when applied to the skin of our volar wrist (where the skin thickness is comparable to the plantar skin of the rat). An increased response to this stimulus is thus indicative of mechano-allodynia (a pain response to a normally innocuous stimulus). A 15 g VFH was used to assess mechano-hyperalgesia. This stimulus evokes a withdrawal response 10–20% of the time in the normal rat and it evokes a barely painful pricking pain sensation when applied to our volar wrist. An increased response to this stimulus is thus indicative of mechano-hyperalgesia (a supernormal pain response to a normally noxious stimulus). Withdrawal responses were counted and expressed as an overall percentage response.

2.6.2 Cold-allodynia—The time course of cold-allodynia was assessed with the acetone drop method. A drop (0.05 ml) of acetone was placed against the center of the plantar hind paw. Responses were graded with the following 4-point scale: 0 = no response; 1 = quick withdrawal, flick or stamp of the paw; 2 = prolonged withdrawal or repeated flicking, and 3 = repeated flicking of the paw with licking directed at the ventral side of the paw. Acetone was applied alternately three times to each paw and cumulative scores were then generated by adding the 6 scores for each rat, the minimum score being 0 and the maximum possible score being 18.

2.6.3 Heat sensitivity—Heat hypersensitivity was assessed by the Hargreaves method (Bennett and Hargreaves, 1990; Hargreaves et al., 1988). Scores were derived by averaging the response latencies of six trials (three per side).

2.7 Axon counts

The saphenous nerve at mid-thigh level from oxaliplatin-treated and vehicle-treated rats (n=4/group) sacrificed at the time of approximate peak pain severity (D35) were examined with the electron microscope as described elsewhere (Jin et al., 2008). A montage of low magnification (550X) photomicrographs was made for the area of the entire nerve and all myelinated A-fibers were counted. For C-fiber counts, at a magnification of 3,100X, we started at the 7 o'clock position on the section and scanned horizontally, photographing every second field until reaching the edge of the section; then moved two fields vertically and resumed the horizontal scan. This was repeated until the entire section had been scanned. With this method the sampled areas is ca. 25% of the total cross-sectional area of the nerve.

2.8 ATF-3 staining

Activating transcription factor-3 (ATF-3), a nuclear marker of neurons with damaged axons (Tsujino et al., 2000), was visualized immunocytochemically as described previously (Jin et al., 2008) in dorsal root ganglia L4 and L5 harvested on D35. Anti-ATF3 primary antibody (Santa-Cruz Biotechnology; Santa Cruz, CA) was diluted 1:500. Cell bodies were visualized with a fluorescent Nissl stain (Invitrogen; Carlsbad, CA; dilution: 1:300). ATF-3 staining in 5 sections from each of 8 oxaliplatin-treated rats sacrificed on D35 was compared to the staining in a rat whose sciatic nerve had been transected 3 days before sacrifice. No staining was present in sections processed without exposure to the primary antibody.

2.9 IENF quantification

IENFs were visualized immunocytochemically as described previously (Jin et al., 2008; Siau et al., 2006; Xiao et al., 2009) in vehicle-treated and oxaliplatin treated rats (n = 8/group) sacrificed at the approximate time of peak pain severity (D35). Antibody directed against protein gene-product 9.5 (PGP9.5; Research Diagnostics; Flanders, NJ) was diluted 1:6400. IENFs in the glabrous hind paw skin were counted by an observer blind as to the animal's

group assignment. One section of skin (8–12 mm long) was analyzed for each rat. A low magnification montage of each section was made and the length (straight line estimate) of the epidermal border was measured. IENF counts are expressed as the number per cm of epidermal border. No staining was present in sections processed without exposure to the primary antibody.

2.10 Nerve conduction velocity

Motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV) were assessed as described elsewhere (Xiao et al., 2011). MNCV was determined on D35–D42 in vehicle-treated and oxaliplatin-treated rats (n = 8/group) with confirmed mechano-allodynia and mechano-hyperalgesia. The compound muscle action potential (CMAP) was recorded via an electrode inserted into the flexor hallucis brevis muscle after stimulation of the muscle's innervation at the levels of the sciatic notch (S1) and the ankle (S2; the tibial nerve at the level of the medial malleolus). Stimulation (0.1 msec pulse; 1.0 Hz) intensity was set at 25% above the level that evoked the maximal CMAP amplitude. The conduction velocity was determined by subtracting the latency to onset of the CMAP from S2 from that of S1 and measuring the distance between the two cathodes.

SNCV was measured in vehicle-treated rats and oxaliplatin-treated rats (n = 7/group) with confirmed mechano-allodynia and mechano-hyperalgesia on D36–D42. The sural nerve, which is 95% sensory axons in rat (Swett et al., 1986, 1991), was stimulated at the ankle and the potential was recorded from the distal end of the transected nerve in the popliteal fossa. Stimulation (0.1 msec pulse; 1.0 Hz) intensity was set at 25% above the level that evoked the maximal amplitude potential. Measurements were obtained for the latencies corresponding to the potential's onset (initial positive inflection) and peak, the duration of the potential, and the potential's peak amplitude. Conduction distance was measured by laying a thread over the course of the nerve between the stimulating electrode's cathode and the recording electrode and the conduction velocities corresponding to the potential's onset and peak were computed.

2.11 Electrophysiological single-fiber recording studies

The procedure was the same as that used in previous work (Xiao and Bennett, 2007, 2008a, 2008b; Xiao et al., 2009). Recordings were made in 5 control animals and 5 oxaliplatin-treated animals on D35–D43. Subcutaneous needle electrodes were inserted across the lateral surface of the ankle for stimulation of sensory afferent axons (sural nerve) innervating the hind paw. Microfilaments were dissected from the distal end of the transected nerve and draped over a silver-wire hook electrode that was referenced to a needle electrode inserted in adjacent muscle. This arrangement records axonal activity originating in the periphery. By gradually increasing the stimulus intensity, we determined the number of individually-identifiable fibers (i.e., those with discreet and constant threshold, latency, and waveform) in each microfilament. The incidence of individually-identifiable fibers with spontaneous discharge (at least 5 impulses in 5 min) was noted, as was their discharge frequency. The conduction velocity was determined by measuring the distance between the stimulation site and the nerve transection. We did not differentiate between A-fibers with conduction velocities in the Aδ and Aβ ranges because it is impossible to differentiate functional classes of A-fibers on this basis (Djouhri and Lawson, 2004). We purposely avoided characterizing the fibers' responses to receptive field stimulation because doing so requires repeated application of noxious stimuli that might sensitize nociceptors. Sensitized nociceptors have an ongoing discharge that would be impossible to distinguish from oxaliplatin-evoked spontaneous discharge. This problem is potentially severe because chemotherapeutics have been shown to induce a sensitization-like phenomenon in at least some nociceptors (Tanner et al., 1998, 2003; Dina et al., 2001).

2.12 Mitochondria counts

Vehicle-treated and oxaliplatin-treated rats (n = 4/group) were sacrificed on D35 and prepared for EM analysis as described above. Random samples of axons and Schwann cells in cross-sections of the saphenous nerve were obtained as follows. At a magnification of 10,200X, we started at the 7 o'clock position and moved horizontally, photographing every third field until the edge of the section was reached, and then moved vertically two fields and resumed the horizontal scan, again photographing every third field. This procedure continued until we had photographed at least 60 A-fibers, 60 myelinating Schwann cells, and 60 C-fibers. The Remak bundle formed by non-myelinating Schwann cells often appears as adjacent groups of several C-fibers embedded in Schwann cell processes separated by extracellular space. Although these groups probably belong to the same Schwann cell, it is impossible to be certain of this and thus difficult to enumerate the number of non-myelinating Schwann cells in a field of view. Thus, for counts of mitochondria in non-myelinating Schwann cells, we continued the sampling procedure until we had photographed a sufficient number of Schwann cell processes to obtain at least 50 mitochondria. Swollen and vacuolated mitochondria were identified as described previously (Jin et al., 2008).

2.13 Acetyl-L-carnitine and olesoxime

We tested acetyl-L-carnitine (ALCAR) and olesoxime, both of which have been shown to ameliorate mitochondrial dysfunction (Bordet et al., 2007, 2008). The drugs were given on the day before the first oxaliplatin injection and then daily for a total of 22 administrations (ALCAR) or 17 administrations (olesoxime). ALCAR was given PO at 100 mg/ml/kg in distilled water. Olesoxime was given PO at 30 mg/ml/kg in corn oil. On those days when both drug and oxaliplatin were administered, drug was given 4 hr prior to oxaliplatin. All behavioral measures were obtained by an observer who was blind as to drug condition. The ALCAR and olesoxime dosing protocols used here were previously shown to be effective against paclitaxel-evoked painful peripheral neuropathy (Flatters et al., 2006; Jin et al., 2008; Xiao et al., 2009; Zheng et al., 2011).

2.14 Statistics

Statistical analyses were carried out with InStat version 3 (GraphPad Software, Inc., La Jolla, CA). Multiple comparisons within an experiment were analyzed with Bonferroni-corrected *t*-tests or Dunnett's *t*-test for multiple comparisons to control. $p < 0.05$ was considered significant.

3 Results

3.1 Oxaliplatin did not affect general health or kidney function

There was a cessation of weight gain during oxaliplatin treatment (Fig. 1). The normal rate of weight gain resumed afterwards, but a small, statistically significant difference from the controls persisted for the duration of the experiment. There were no changes in the appearance of the animals and there were no deaths. Indices of kidney function (Table 1) were normal in animals assessed on D7 and D35.

3.2 Behavioral assays

3.2.1 Time course of mechano-allodynia—The oxaliplatin-treated animals had a significantly greater response frequency to the 4 g VFH stimulus on D8 (Fig. 2A). The severity of mechano-allodynia increased gradually, reached peak severity on D35 (4 weeks after the last oxaliplatin injection), gradually decreased over the following five weeks, and

returned to control levels on D105. There was no significant change in response frequency in the vehicle-injected control group.

3.2.2 Time course of mechano-hyperalgesia—A statistically significant increase in response frequency to the 15 g VFH stimulus was present on D8 (Fig. 2B). The severity of mechano-hyperalgesia increased gradually, reached peak severity on D22 (2.5 weeks after the last oxaliplatin injection), remained at about the peak level until D73, and returned to control levels by D105. There was no significant change in response frequency to the 15 g stimulus in the control group.

3.2.3 Time course of cold-allodynia—Statistically significant hypersensitivity to cold was present on D7 and D14 (Fig. 2C). The severity of cold-allodynia increased to a peak at D22 (2.5 weeks after the last oxaliplatin injection), remained at about this level until D56, decreased sharply by D73, and returned to control levels by D105. The vehicle-injected control animals had a relatively small but statistically significant increase in sensitivity to cold. Nevertheless, the increase seen for the oxaliplatin-treated group was significantly greater than the control group's increase at all time points.

3.2.4 Heat-sensitivity—There were no statistically significant differences between the control and oxaliplatin groups in their responses to heat at any time point (D8–D42) (Fig. 2D).

3.3 Oxaliplatin did not cause degeneration of peripheral nerve axons

We saw no degenerating A-fibers or C-fibers. The numbers of A-fibers and C-fibers in the nerves from oxaliplatin-treated and vehicle-treated groups were nearly identical (Fig 3A,B).

3.4 Oxaliplatin causes a partial loss of intraepidermal nerve fibers (IENFs)

Vehicle-injected control animals had 338 ± 24.1 (mean ± SEM) IENFs per cm of epidermal border. Oxaliplatin-treated rats had 273.6 ± 26.1 IENFs per cm. This is a statistically significant decrease of 21% (Fig. 3C).

3.5 Oxaliplatin did not induce ATF-3 in DRG neurons

As expected, about 50% of the large and small neurons had ATF-3-positive nuclei in the DRG of the sciatic nerve transection rat (this is the percentage of L4–L5 cells whose axons travel in the sciatic nerve). We did not find a single ATF-3-positive DRG cell nucleus in the L4–L5 DRGs of the oxaliplatin-treated rats (Fig. 3D).

3.6 Oxaliplatin treatment had no effect on motor nerve conduction velocity (MNCV) but slowed sensory nerve conduction velocity (SNCV)

The MNCV in the vehicle-treated and oxaliplatin-treated rats were not significantly different (mean ± SEM): 49.8 ± 2.3 m/s *vs*. 50.6 ± 1.4 m/s, respectively.

The SNCV were slower in the oxaliplatin-treated rats (Table 2), as evidenced by statistically significant increases in the conduction velocities for the onset of the potential (22%) and for the potential's peak (14%). However, there were no significant effects on the potential's duration or peak amplitude.

3.7 Abnormal spontaneous discharge

We recorded 141 A-fibers and 70 C-fibers from 34 microfilaments in 5 control animals. None of these fibers had any spontaneous discharge (Table 3). We recorded 126 A-fibers and 67 C-fibers from 36 microfilaments in 5 oxaliplatin-treated animals. A significant

increase in the incidence of fibers with spontaneous discharge was present in the oxaliplatin-treated rats (15% of the A-fibers and 34% of the C-fibers). Both the A-fiber and the C-fiber spontaneous discharge was slow (less than 2.0 Hz) and had a distinctly irregular pattern.

3.8 Structural change in mitochondria

Swollen and vacuolated mitochondria were found in A-fibers and C-fibers in both vehicle-treated and oxaliplatin-treated groups, but were significantly more common after chemotherapy (Fig. 4A,B). In vehicle-treated animals, A-fibers and C-fibers contained 33.8 ± 1.6% (total number of mitochondria: n = 1632) and 22.3 ± 4.2% (n = 451) swollen and vacuolated mitochondria, respectively. In oxaliplatin-treated animals, the A-fibers had 46.4 ± 2.1% (n = 1537) swollen and vacuolated mitochondria and the C-fibers had 56.2 ± 1.0% (n = 428). The oxaliplatin-evoked increases are statistically significant for both A-fibers and C-fibers (Fig. 4C).

Swollen and vacuolated mitochondria were relatively rare in the cytoplasm of myelinating Schwann cells in both vehicle-treated and oxaliplatin-treated animals: 6.2 ± 1.5 (n = 1331) and 6.2 ± 1.5 (n = 1192), respectively. The same was true in the cytoplasm of the non-myelinating Schwann cells: vehicle-treated: 2.0 ± 2.4 (n = 245); oxaliplatin-treated: 2.4 ± 0.5 (n = 246). The between-group differences for Schwann cell mitochondria are not statistically significant (Fig. 4D).

3.9 Effects of prophylactic treatment with ALCAR and olesoxime

Oxaliplatin-treated rats treated with vehicle injections developed the expected mechano-allodynia and mechano-hyperalgesia (Fig. 5). Prophylactic treatment with ALCAR produced a partial but statistically significant reduction in the severity of both mechano-allodynia and mechano-hyperalgesia (area-under-the-curve values: 51% and 55% reductions, respectively). This effect was permanent in the sense that it remained long after the last dose of ALCAR.

Prophylactic treatment with olesoxime (Fig. 6) also produced a partial but statistically significant reduction in the severity of both mechano-allodynia and mechano-hyperalgesia (area-under-the-curve values: 58% and 53% reductions, respectively), and these reductions also remained long after the last dose of olesoxime.

4 Discussion

4.1 Comparison to the clinic

Our observations indicate that the oxaliplatin treatment protocol used here in the rat produces a chronic painful peripheral neuropathy like that seen in the clinic. The animals have mechano-allodynia, mechano-hyperalgesia, and cold-allodynia; the patients have the same symptoms (Binder et al., 2007; Cata et al., 2006). The rats did not develop heat hypersensitivity; clinically, decreases in the heat-pain threshold are absent or relatively minor (Binder et al., 2007; Cata et al., 2006). The delay between treatment termination and the appearance of sensory dysfunction and the gradual worsening of symptom severity that are often seen in the patient are also seen in the animal. As in the patient, there is evidence of SNCV slowing without any change in MNCV.

Oxaliplatin caused a partial, but statistically significant, loss of somatosensory terminal receptor arbors (IENFs) without causing any degeneration of peripheral nerve axons and without activating the ATF-3 signal in DRG cell bodies. Oxaliplatin evoked a clearly abnormal incidence of spontaneously discharging A-fibers and C-fibers. We are not aware of any comparable anatomical or electrophysiological data from oxaliplatin-treated patients.

The sensory abnormalities have a delayed onset and persist for long after the cessation of drug treatment; thus the effects produced by this model are clearly due to the chronic, rather than the acute, oxaliplatin-evoked neuropathy. We suggest that this model will be of use in studies seeking to understand the mechanism of oxaliplatin neurotoxicity and in studies aimed at finding drugs to prevent or control it.

4.2 Effects on general health and kidney function

The rats stopped gaining weight during oxaliplatin treatment, but the normal rate of weight gain resumed thereafter. No rats died during or after the oxaliplatin treatment protocol used here. In contrast, studies in rats that have used higher oxaliplatin doses have reported fatalities (Cavaletti et al., 2001; Holmes et al., 1998; Jamieson et al., 2005; Ling et al., 2007a).

Indices of kidney function in oxaliplatin-treated rats did not differ significantly from the control group. We thus conclude that our results can not be confounded by a uremic neuropathy. We believe that it is essential to assess kidney function in animals receiving platinum-complex agents because platinum is nephrotoxic and can lead to uremic polyneuropathy, which is a distal, symmetrical, predominately sensory peripheral neuropathy (Krishnan and Kiernan, 2007) that would be difficult or impossible to differentiate from the effect of the chemotherapeutic agent itself. In the clinic, the recommended dosage of oxaliplatin does not produce kidney dysfunction, although there is evidence for sub-clinical damage to proximal tubular cells (Haschke et al., 2010). However, oxaliplatin's effect on the kidney is evident when treating patients with pre-existing renal disease (Labaye et al., 2005; Massari et al., 2000). In studies with animals it is difficult to determine a dose that is equivalent to the clinically safe dose and thus one can not simply presume that kidney damage is absent. Rat studies using cumulative oxaliplatin doses of 30–48 mg/kg have reported mortality during and after the dosing regimen, and abdominal swelling (ascites accumulation) in both the dying animals and in survivors (Cavaletti et al., 2001; Holmes et al., 1998; Jamieson et al., 2005; Ling et al., 2007a). The reason for this has not been determined, but it may be related to nephrotoxicity. A potential confound with uremic neuropathy is especially problematic with studies using cisplatin, which is much more nephrotoxic than oxaliplatin in both man and rat (Pispirigos et al., 1993; Safirstein et al., 1986).

4.3 Axon counts, ATF-3 staining, and IENF degeneration

Oxaliplatin treatment did not result in degeneration of peripheral nerve axons and did not evoke the ATF-3 signal in the DRG neurons. The lack of the ATF-3 response is consistent with the absence of peripheral nerve axon degeneration. Similarly, our paclitaxel treatment protocol does not cause degeneration of peripheral nerve axons or the ATF-3 signal (Bennett et al., 2011; Flatters and Bennett, 2006).

However, oxaliplatin did produce a significant loss (21%) of IENFs. The amount of oxaliplatin-evoked IENF degeneration is comparable to what has been seen in most prior studies with paclitaxel: 24% (Siau et al., 2006), 24% (Jin et al., 2008), 46% (Xiao et al., 2009), and 21% (Bennett et al., 2011). For paclitaxel, IENF degeneration without degeneration of peripheral nerve axons has been ascribed to differential sensitivity to mitotoxicity between the sensory receptor arbor and its parent axon (Bennett et al., 2011). The same may be true for oxaliplatin.

IENF degeneration and neuropathic pain appear to be linked. In paclitaxel-treated rats, both have about the same delays to onset and peak, and in oxaliplatin-treated and paclitaxel-treated rats, drugs that block the development of the pain also protect against IENF

degeneration (Flatters et al., 2006; Jin et al., 2008; Xiao et al., 2009; Bennett et al., 2011; Boyette-Davis et al., 2011; Meyer et al., 2011; Zheng et al., 2011).

4.4 Nerve conduction

Patients with oxaliplatin-evoked chronic sensory peripheral neuropathy have slowed conduction in sensory, but not motor, axons (Argyriou et al., 2008; Krishnan et al., 2005; Pietrangeli et al., 2006). We found the same in the rat. The degree of slowing (22%) is within the range of slowing (ca. 6–27%) reported in other animal studies of oxaliplatin (Cavaletti et al., 2001; Jamieson et al., 2005; McKeage et al., 2001). Meyer et al. (2011) report an oxaliplatin-evoked 24% decrease in SNCV but they also see a decrease in the amplitude's peak potential. Rats treated with paclitaxel have normal MNCV and normal SNCV (Xiao et al., 2011)

It is noteworthy that in the rat, at least, oxaliplatin-evoked SNCV slowing can not be due to demyelination or degeneration of peripheral nerve axons because these were shown to be absent. IENF degeneration can also not be the cause because the nerve potential is evoked by subcutaneous electrical stimulation of the nerve, bypassing the IENFs. Clinically, conduction slowing due to peripheral nerve degeneration or demyelination is accompanied by a change in the potential's shape – the peak amplitude is decreased and the duration increases, sometimes with the appearance of multiple small peaks. We found slowing but no change in the potential's shape, peak amplitude or duration. The mechanism that produces slowed conduction in sensory axons in the absence of axonal degeneration or demyelination is unknown. It is also not known whether slowing in the absence of degeneration has any sensory consequences.

4.5 Oxaliplatin-evoked neuropathic pain

Oxaliplatin evoked mechano-allodynia, mechano-hyperalgesia and cold-allodynia, each of which lasted for about 2.5 months after the last dose of oxaliplatin. For mechano-allodynia and mechano-hyperalgesia, there was a distinctly gradual increase in pain severity over a period of two weeks or more after the last dose of oxaliplatin. For mechano-allodynia, mechano-hyperalgesia and cold allodynia, peak pain severity appeared with distinct delays of 2.5–4.0 weeks after the last dose of oxaliplatin. Similarly for paclitaxel, mechano-allodynia, mechano-hyperalgesia, and cold allodynia appear with a distinct delay to onset, delay to peak severity, and duration of 3 months or more (Bennett et al., 2011; Flatters and Bennett, 2004; Flatters and Bennett, 2006; Flatters et al., 2006; Polomano et al., 2001; Xiao et al., 2009).

It is noteworthy that oxaliplatin did not alter sensitivity to noxious heat. Heat hypersensitivity is also absent, or relatively minor, in oxaliplatin-treated patients and in patients treated with other chemotherapeutics (Binder et al., 2007; Cata et al., 2006; Dougherty et al., 2004, 2007). However, paclitaxel does evoke heat-hyperalgesia (Chen et al., 2011; Flatters and Bennett, 2006; Polomano et al., 2001). This is the only significant difference between oxaliplatin-induced and paclitaxel-induced neuropathies that we have seen. Recent studies indicate that oxaliplatin-evoked mechano- and cold-hypersensitivity are mediated by sensitization of the transient receptor potential ankyrin 1 (TRPA1) channel, while there is little or no oxaliplatin effect on the transient receptor potential vanilloid 1 (TRPV1) channel (Nassini et al., 2011). TRPA1 is known to be involved in mechano- and cold-hypersensitivity states, while TRPV1 is known to be involved in heat-hypersensitivity. Thus, oxaliplatin's effect on TRPA1 and the absence of effect on TRPV1 is consistent with the presence of mechano- and cold- hypersensitivity and the absence of heat-hypersensitivity. TRPA1 has also been implicated in paclitaxel-evoked mechano- and cold-hypersensitivity (Chen et al., 2011; Moran et al., 2011), but paclitaxel also sensitizes the

TRPV1 channel and this may account for paclitaxel-evoked heat-hyperalgesia (Chen et al., 2011). Ta et al. (2009) have shown that in mice heat-hyperalgesia is not present with oxaliplatin treatment but is present after cisplatin. Moreover, it has been shown that the cisplatin-induced heat-hyperalgesia is related to TRPV1 function (Ta et al., 2010). The reason for the difference between oxaliplatin and cisplatin is unknown; one would have expected them to be very similar because the platinum ion is the neurotoxic agent for each.

4.6 Prior studies of oxaliplatin-induced neuropathic pain

We are aware of four prior studies that give detailed characterizations of the chronic neuropathic pain produced by cumulative exposure to oxaliplatin in the rat (Joseph et al., 2008; Ling et al., 2007a; Sakurai et al., 2009; Meyer et al., 2011) and two such studies in the mouse (Ta et al., 2009; Renn et al., 2011). All of the chronic studies used different single dose amounts, different cumulative doses, and different dosing schedules. It is thus difficult to make comparisons because clinical experience suggests that each of these variables may be important for the induction of the neuropathy (Argyriou et al., 2008; Cersosimo, 2005; Quasthoff and Hartung, 2002; Windebank and Grisold, 2008). Ling et al. (2007a) assessed pain sensitivity for up to 21 days after the last exposure to oxaliplatin, at which time they found significant mechano-allodynia, mechano-hyperalgesia, and cold allodynia, and equivocal evidence for heat-hypersensitivity. However they did not see any increase in symptom severity after dosing was halted. Meyer et al. (2011) detected mechano-allodynia, mechano-hyperalgesia, and cold-allodynia of delayed onset, each of which lasted for the entire observation period (9–11 days after the last oxaliplatin injection). As we report here, these authors also found no evidence for heat-hyperalgesia. In the mouse, Ta et al. (2009) found mechano-allodynia and cold-allodynia, but no heat hypersensitivity, which lasted for at least six weeks after oxaliplatin treatment. Several studies have used repeated dosing protocols but have measured behavior for only a few days after the last injection; this is too short a time to determine whether the symptoms are due to the acute or to the chronic neuropathy (Jospeh et al., 2008; Renn et al., 2011; Sakurai et al., 2009).

Studies of the effects of a single injection of oxaliplatin on pain sensitivity would seem to be relevant to oxaliplatin's acute neuropathy. Such studies have shown mechano-allodynia (v. Frey hair test) and cold-allodynia lasting for a few days (Ling et al., 2007b, 2008). Joseph and colleagues (2008). Joseph and colleagues (2009) report the additional presence of heat-hyperalgesia and mechano-hyperalgesia (paw pressure test). The mechano-hyperalgesia was reported to last for at least 40 days, which seems far too long for oxaliplatin's acute reaction. However, a more recent study suggests that it lasts for 12 days or less (Alvarez et al., 2011). Ling et al. (2007b, 2008) did not find any mechano-hyperalgesia (paw pressure test) or heat hyperalgesia after a single injection of oxaliplatin.

4.7 Abnormal spontaneous discharge

There was a clearly abnormal incidence of A-fibers and C-fibers with spontaneous discharge of low frequency and irregular pattern in the oxaliplatin-treated rats. A prior study in paclitaxel-treated rats (Xiao and Bennett, 2008a) found a very similar incidence of spontaneously active fibers with the same low frequency and irregular discharge pattern is seen in rats with paclitaxel-evoked painful peripheral neuropathies (see Table 3). There is evidence suggesting that the spontaneous discharge seen in both oxaliplatin and paclitaxel-treated rats is linked to mitotoxicity (Xiao and Bennett, 2011).

It is likely that the spontaneous discharge is in some way associated with the appearance of allodynia and hyperalgesia, although the exact mechanism(s) responsible for the pain hypersensitivity is not known. The percentages of fibers with spontaneous discharge is substantial, but the mean discharge frequency is low (1–2 Hz). The perceptual consequences

of such low frequency discharge is not clear, but it is noteworthy that a comparably low discharge frequency in A-fibers and C-fibers is found in a chronic inflammatory pain condition (Xiao and Bennett, 2007; 2008b)

4.8 Mitochondrial changes

Oxaliplatin caused a significant increase in the incidence of swollen and vacuolated mitochondria in A-fibers and C-fibers: 37.3% and 152% relative to vehicle control, respectively. These increases are similar to those found in prior studies with paclitaxel: A-fibers: 38.8% and 60.7% (Jin et al., 2008; Xiao et al., 2011); C-fibers: 72.9% and 111.8% (Jin et al., 2008; Xiao et al., 2011). Oxaliplatin did not cause mitochondrial swelling and vacuolation in myelinating and non-myelinating Schwann cells; neither does paclitaxel (Xiao et al., 2011). As noted in prior studies, A-fibers and C-fibers in normal animals have a surprisingly high incidence of swollen and vacuolated mitochondria. There is evidence showing that swelling and vacuolation may be associated with mitochondrial stress at the time of aldehyde exposure (Brewer and Lynch, 1986). Nevertheless, the pathophysiological significance of the chemotherapy-evoked increase is ambiguous. It is thus important that recent work (Zheng et al., 2011) shows that peripheral nerve mitochondria from rats with oxaliplatin-induced and paclitaxel-induced painful peripheral neuropathy have very similar deficits in Complex I-mediated and Complex II-mediated mitochondrial respiration and very similar deficits in stimulated ATP production.

Chemotherapy-evoked mitotoxicity and the subsequent energy deficiency may be the cause of IENF degeneration and abnormal spontaneous discharge (for discussion see Bennett et al, 2011 and Xiao and Bennett, 2011). A chemotherapy-induced mitochondrial injury in primary afferent neurons may also be the initial event leading to mechano- and cold-hypersensitivity. Mitochondrial dysfunction is expected to cause an increase in the release of free electrons from the electron transport system and subsequent oxidative and nitrative stress. There is evidence that this occurs with the oxaliplatin and paclitaxel treatment protocols used here (Doyle et al., 2011; Xiao and Bennett, 2011). The TRPA1 channel is known to respond to increased levels of oxidative stress and Nassini et al. (2011) have shown that oxidative stress is a likely cause of oxaliplatin-induced TRPA1 sensitization. Treatment with auranofin, which increases oxidative stress, worsens oxaliplatin-induced and paclitaxel-induced mechano-hypersensitivity and peroxynitrite decomposition catalysts inhibit paclitaxel-induced mechano-hypersensitivity (Doyle et al., 2011). However, a recent report suggests that sensitization of the primary afferent TRPM8 channel, rather than TRPA1, may be the cause of oxaliplatin-evoked cold-allodynia (Descourers et al., 2011).

4.9 ALCAR and olesoxime

ALCAR was examined because of its well known ability to improve the functional status of mitochondria in neurons from aged brain (reviewed in (Ames and Liu, 2004)) and in neurons that have been damaged by mitochondrial toxins (Virmani et al., 2005). The prophylactic ALCAR treatment protocol significantly reduced the development of oxaliplatin-evoked pain and the effect persisted long after ALCAR administration. Ghirardi et al. (2005) have also shown the protective effects of prophylactic ALCAR treatment against oxaliplatin neuropathy. The same ALCAR prophylactic treatment protocol inhibits the development of paclitaxel-evoked pain (Flatters et al., 2006; Jin et al., 2008). Moreover, the same ALCAR treatment prevents both oxaliplatin-evoked and paclitaxel-evoked deficits in mitochondrial function (Zheng et al., 2011).

Olesoxime was examined because it has neuroprotective and pro-regenerative effects that are believed to result from an exclusively mitochondrial site(s) of action (Bordet et al., 2007, 2008). The prophylactic olesoxime treatment protocol significantly reduced the development

of oxaliplatin-evoked pain and the effect persisted long after olesoxime administration. The same olesoxime treatment protocol inhibits the development of paclitaxel-evoked pain (Xiao et al., 2009).

A recent report shows that the neurosteroid, allopregnanolone, prevents and reverses many of the phenomena associated with oxaliplatin's painful peripheral neuropathy (Meyer et al., 2011). The mechanism of action for the effect is unknown and neurosteriods have very many actions; however, allopregnanolone, like ALCAR and olesoxime, is known to protect mitochondria (Sayeed et al., 2009).

4.10 Oxaliplatin compared to paclitaxel

Oxaliplatin and paclitaxel have very different anti-cancer mechanisms of action, but both produce a chronic, distal, bilaterally symmetrical, sensory peripheral neuropathy that is often accompanied by neuropathic pain. Our results show that the chronic peripheral neuropathies induced by these two drugs are very similar and suggest that there is very considerable overlap in their pathophysiological mechanisms. Nearly all of the oxaliplatin-evoked effects reported here have also been seen in prior work with paclitaxel-evoked painful peripheral neuropathy. There are only two exceptions. The first of these, slowing of SNCV produced by oxaliplatin but not by paclitaxel, is probably irrelevant to the sensory abnormalities and thus of little interest. The second, heat-hyperalgesia evoked by paclitaxel but not by oxaliplatin, appears to involve differential effects on TRPA1 (or TRPM8) and TRPV1 channel sensitization; this is clearly of clinical significance.

Prior work with the paclitaxel model led to the proposal that a toxic effect on mitochondria in primary afferent neurons leads to a deficit in axonal energy supply that is the fundamental cause of the chronic sensory neuropathy's symptoms (Bennett et al., 2011; Flatters and Bennett, 2006; Xiao et al., 2011). The data presented here and studies of oxaliplatin's effects on axonal mitochondrial function (Zheng et al., 2011) point to the same conclusion for oxaliplatin. This suggests that mitotoxicity is the core mechanism for both drugs. It is possible that other chemotherapeutics have mitotoxicity as a core mechanism for peripheral neuropathy. For example, we have observed that rats with chronic painful peripheral neuropathy due to the proteasome inhibitor, bortezomib, have anatomical and functional abnormalities of axonal mitochondria that are very similar to those seen with oxaliplatin and paclitaxel (unpublished observations). We note that the hypothesis that mitotoxicity is a core pathophysiology for chemotherapy-induced peripheral neuropathy does not posit that the various chemotherapeutic agents damage mitochondria in the same way; indeed this seems highly unlikely. The presence of a core pathophysiological mechanism suggests that drugs aimed at preventing or controlling the painful peripheral neuropathy produced by one chemotherapeutic agent may be effective in all.

It might be argued that clinical evidence for the involvement of motor signs differentiates oxaliplatin-induced and paclitaxel-induced chronic peripheral neuropathy. There are claims of distal motor axon abnormality in some (but not all) patients with chronic sensory neuropathy following paclitaxel or docetaxel treatment. These claims are either unsubstantiated (Augusto et al., 2008) or come from studies of patients who had concurrent or prior exposure to cisplatin and whose pre-treatment motor function was not established (reviewed in Argyriou et al., 2008). Even if it is true that taxanes sometimes affect the distal motor axon, this would not negate the possibility that paclitaxel and oxaliplatin share a core pathophysiological mechanism. The idea that oxaliplatin and paclitaxel (and perhaps other chemotherapeutics) share a core mechanism for the production of chronic sensory peripheral neuropathy is not incompatible with the possibility that they also produce other neurological effects with different pathophysiological mechanisms. For example, both oxaliplatin and paclitaxel produce acute neuropathies that are distinct from each other and distinct from the

chronic sensory neuropathy (Argyriou et al., 2008; Cersosimo, 2005; Loprinzi et al., 2011). The idea of a shared core pathophysiology is also not incompatible with the possibility that higher doses of either agent might produce additional neurotoxic effects, either via the common mechanism or via different mechanisms.

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Abbreviations

ALCAR	acetyl-L-carnitine
ATF-3	activating transcription factor 3
BUN	blood urea nitrogen
CIPN	chemotherapy-induced peripheral neuropathy
CMAP	compound muscle action potential
DRG	dorsal root ganglion
EM	electron microscopy
IENF	intraepidermal nerve fiber
MNCV	motor nerve conduction velocity
NAG	N-acetyl-β-D-glucosaminidase
PGP9.5	protein gene-product 9.5
SNCV	sensory nerve conduction velocity
TRPA1	transient receptor potential ankyrin 1 channel
TRPM8	transient receptor potential melastatin 8 channel
TRPV1	transient receptor potential vanilloid 1 channel
VFH	v. Frey hair

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- Oxaliplatin evokes a chronic painful peripheral sensory neuropathy in rat
- Oxaliplatin induces abnormal spontaneous discharge in A-fibers and C-fibers
- Oxaliplatin induces swelling and vacuolation in axonal mitochondria
- IENF degeneration is present without degeneration of peripheral nerve axons
- Oxaliplatin-induced effects are very similar to those induced by paclitaxel

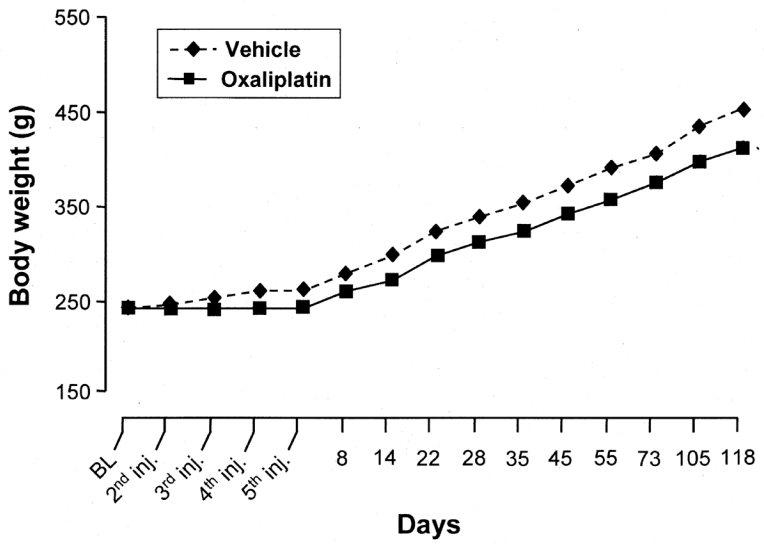

Fig. 1.

Body weights for vehicle-treated and oxaliplatin-treated rats used in the behavioral time-course assays for mechano-allodynia and mechano-hyperalgesia (see next figure). Mean ± SEM (error bars are smaller than the symbols); n = 12/group. BL: Baseline weight on the day of the first injection. The difference between groups is statistically significant from the day of the 4th injection onwards (two-way ANOVA followed by Bonferroni-corrected *t*-tests for each time point).

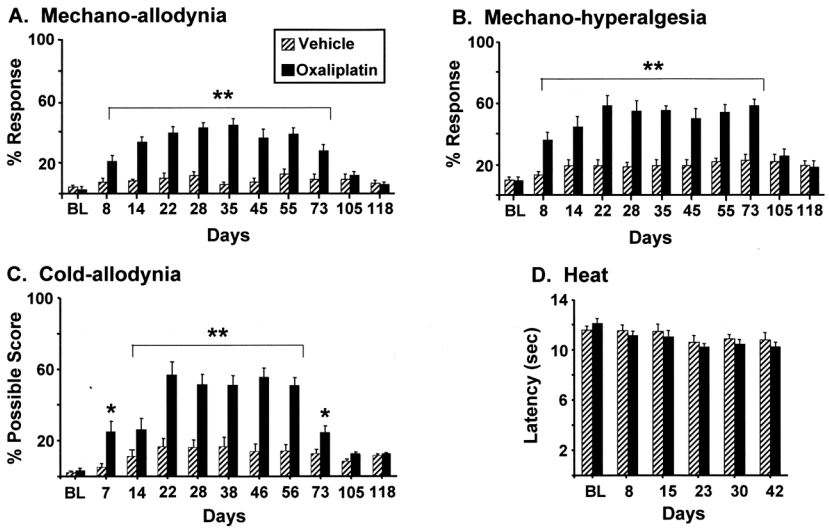

Fig. 2.

Time courses for oxaliplatin-evoked (**A**) mechano-allodynia, (**B**) mechano-hyperalgesia, (**C**) cold-allodynia, and (**D**) heat sensitivity. **A** and **B** are the same animals (n = 8/group), **C** and **D** are separate groups (n = 12 and n = 9, respectively). The last oxaliplatin injection was on D4. Note the delays to peak pain severity for mechano-allodynia, mechano-hyperalgesia, and cold-allodynia. Oxaliplatin treatment had no effect on heat pain thresholds. BL: Baseline test before the first injection. Means ± SEM. *, ** $p < 0.05$, < 0.01 (repeated measures ANOVA with Dunnett's test for *post hoc* pair-wise comparisons to pre-injection baseline). There were no significant variations over time for the vehicle-treated groups in A, B, and D. However, the vehicle-treated group in C had a small but statistically significant increase in sensitivity to cold; the reason for this is not known. Nevertheless, the oxaliplatin-treated group was significantly more sensitive in comparison to the vehicle-treated group (Bonferroni-corrected *t*-tests for all time points).

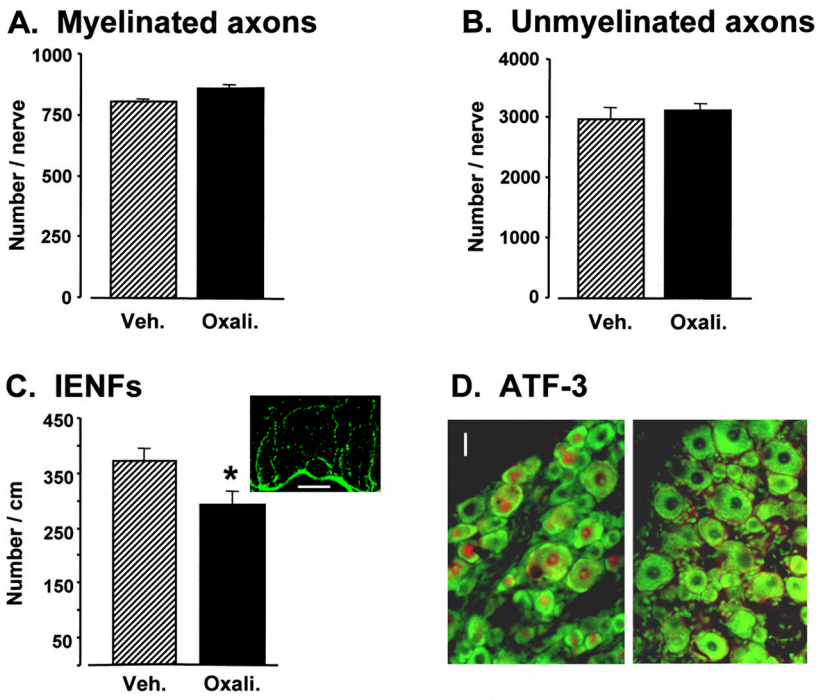

Fig. 3.

Anatomy. Counts of peripheral nerve A-fibers (**A**) and C-fibers (**B**) in vehicle-treated and oxaliplatin-treated rats (n = 4/group) sacrificed on D35. Means ± SEM. The between group differences are not statistically significant (*t*-tests). (**C**) Counts of intraepidermal nerve fibers (IENF) per cm of epidermal border in vehicle-treated and oxaliplatin-treated rats. Oxaliplatin treatment evoked a significant loss of IENFs. * $p < 0.05$ (*t*-test). Inset: PGP9.5-stained IENFs in the skin of the plantar hind paw of an oxaliplatin-treated rat. IENFs arise from subepidermal nerve fascicles, cross the epidermal basal lamina, and form the afferent's terminal receptor arbor by branching within the epidermis and issuing *en passant* and terminal receptor boutons. (**D**) Sections from the L5 DRG from a rat with an ipsilateral sciatic nerve transection (left) and from an oxaliplatin-treated rat (right). ATF-3-positive nuclei are stained red, Nissl substance is stained green. Scale bar = 30 μm. No ATF-3-positive DRG cells were found in oxaliplatin-treated rats.

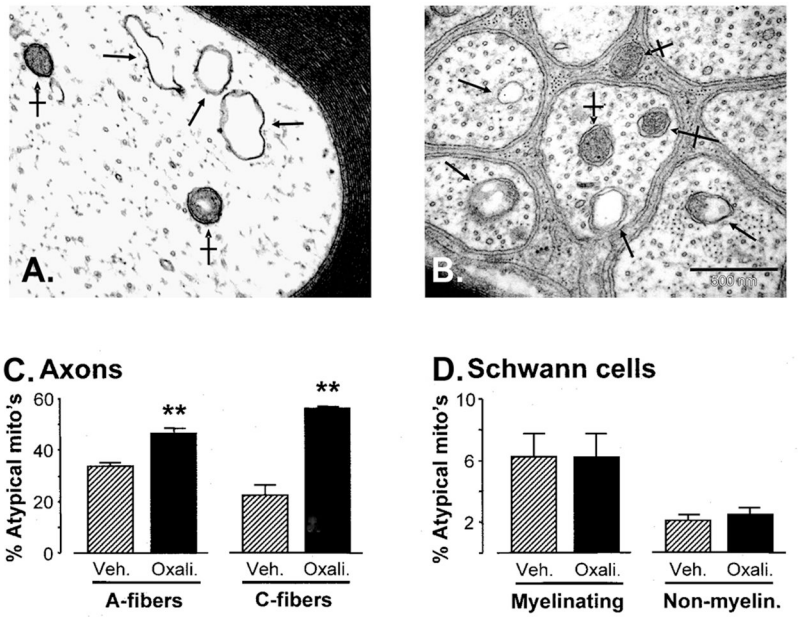

Fig. 4.

Atypical (swollen and vacuolated) mitochondria in A-fibers and C-fibers and their respective Schwann cells in vehicle-treated and oxaliplatin-treated rats. (**A**) Portion of a myelinated axon from an oxaliplatin-treated rat. As shown here, axons usually had a mixture of normal (barred arrows) and swollen and vacuolated (plain arrows) mitochondria. (**B**) Portion of a Remak bundle from an oxaliplatin-treated rat showing C-fibers with normal (barred arrows) and swollen and vacuolated (plain arrows) mitochondria. The uppermost barred arrow points to a normal mitochondrion in the Schwann cell's cytoplasm. Scale bar = 0.5 μm. (**C**) The percentage (means ± SEM) of swollen and vacuolated mitochondria in A-fiber and C-fiber axons in oxaliplatin-treated rats was significantly greater than in vehicle-treated rats. ** $p < 0.01$ (*t*-tests). (**D**) The percentage (means ± SEM) of swollen and vacuolated mitochondria in the cytoplasm of myelinating and non-myelinating (i.e., Remak bundle) Schwann cells in vehicle-treated and oxaliplatin-treated rats were low and not significantly different (*t*-tests).

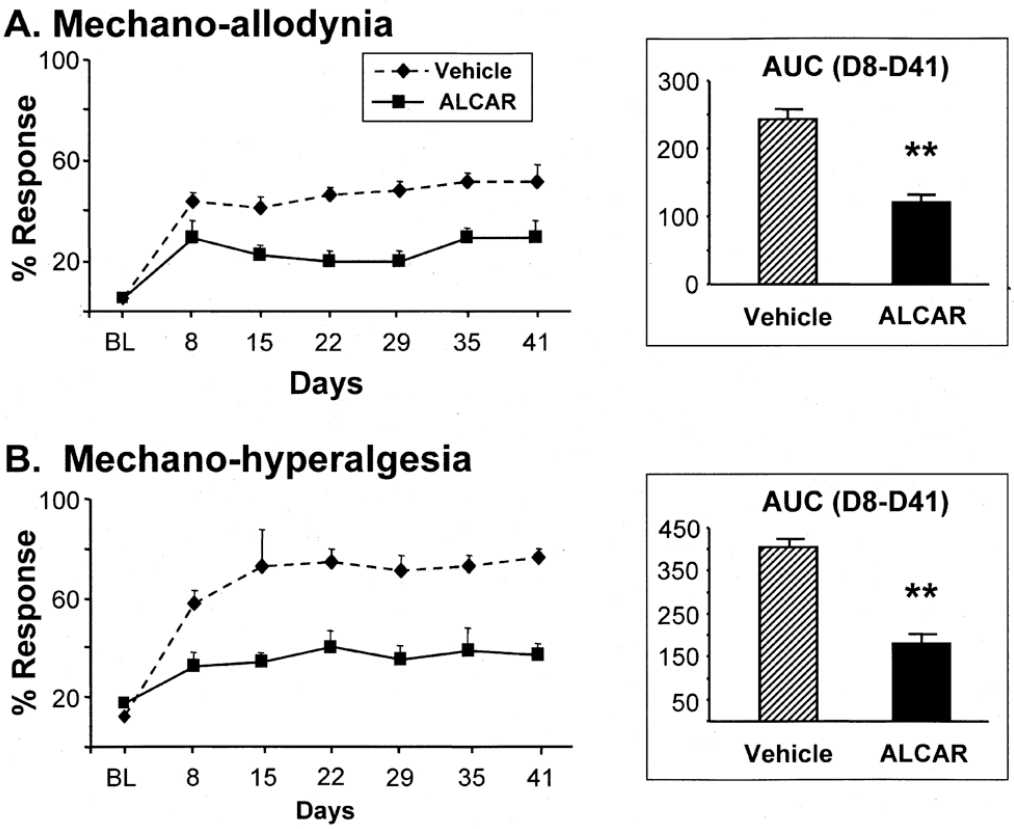

Fig. 5.

Effects of acetyl-L-carnitine (ALCAR) on oxaliplatin-evoked painful peripheral neuropathy. The vehicle-treated group developed the expected statistically significant mechano-allodynia (**A**) and mechano-hyperalgesia (**B**). ALCAR treatment significantly reduced the severity of mechano-allodynia and mechano-hyperalgesia and the effect persisted for at least three weeks after the last ALCAR injection. **Right:** AUC: area-under-the-curve values. ** $p <$ 0.001 (*t*-test).

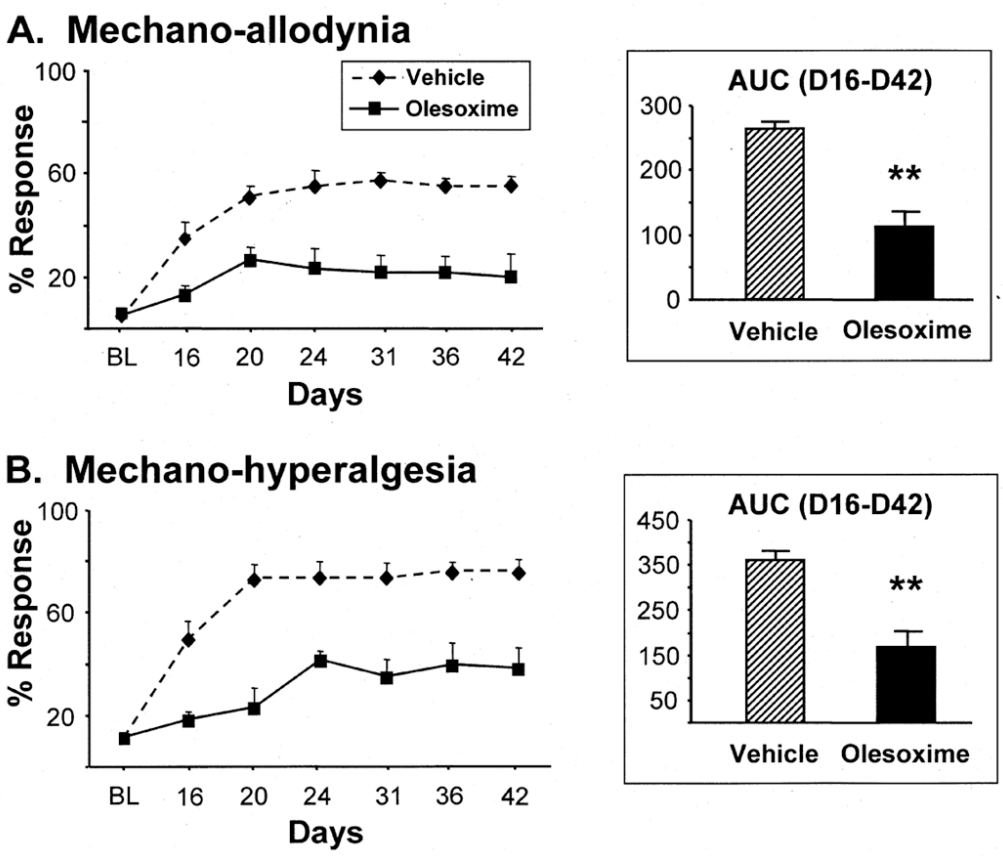

Fig. 6.

Effects of olesoxime on oxaliplatin-evoked painful peripheral neuropathy. The vehicle-treated group developed the expected statistically significant mechano-allodynia (**A**) and mechano-hyperalgesia (**B**). Olesoxime treatment significantly reduced the severity of mechano-allodynia and mechano-hyperalgesia and the effect persisted for at least three weeks after the last olesoxime injection. **Right:** AUC area-under-the-curve values. ** $p <$ 0.001 (*t*-test).

Table 1

Oxaliplatin effects on indices of kidney function.

Group	BUN (mg/dL)	Creatinine (mg/dL) Plasma [Urine]	NAG (U/L)	Protein (mg/ml)
Control	22.2 ± 8.6	0.5 ± 0.0 [92.2 ± 3.2]	22.6 ± 3.1	2.5 ± 0.2
Oxaliplatin D7	23.2 ± 7.4	0.4 ± 0.1 [76.2 ± 3.4]	18.0 ± 2.7	1.9 ± 0.2
Oxaliplatin D35	21.7 ± 20.2	0.5 ± 0.1 [94.7 ± 4.2]	23.7 ± 5.3	3.0 ± 0.3

BUN: blood urea nitrogen; Creatinine in plasma and urine; NAG: N-acetyl-β-D-glucosaminidase in urine; Protein in urine. Means ± SEM; N = 10–12/group. None of the oxaliplatin groups' values are significantly different from control (Dunnett's *t*-tests).

Table 2

Oxaliplatin effects on sensory nerve conduction.

Group	Onset (m/s)	Peak (m/s)	Duration (msec)	Amplitude (mV)
Control	75 ± 17.9	36.3 ± 6.4	1.68 ± 0.3	45.8 ± 4.3
Oxaliplatin	59.3 ± 6.1*	31.4 ± 1.9*	1.49 ± 0.3	48.4 ± 3.8

Means ± SEM; n = 7/group.

* $p < 0.05$ relative to control (*t*-tests).

Table 3

Chemotherapy-evoked spontaneous discharge in primary afferent axons.

	Vehicle	Oxaliplatin	Paclitaxel *
A-fibers:			
Incidence of spontaneous discharge (N)	0% (141)	15% (126)	17% (150)
Discharge freq. mean ± SEM (range)	n/a	1.36 ± 0.47 Hz (1.03 – 1.8)	1.6 ± 1.9 Hz (0.03 – 5.9)
C-fibers:			
Incidence of spontaneous discharge (N)	0% (70)	34% (67)	20% (45)
Discharge freq. mean ± SEM (range)	n/a	1.06 ± 0.83 Hz (0.1 – 2.5)	1.15 ± 0.4 (0.4 – 2.0)

* Paclitaxel data from Xiao and Bennett (2008a)

Means ± SEM. N = number of axons studied. n/a: not applicable.