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CHARACTERIZATION OF OXALIPLATIN-INDUCED CHRONIC PAINFUL PERIPHERAL NEUROPATHY IN THE RAT AND COMPARISON WITH 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 with 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. Electron microscopy (EM) analyses found no evidence for axonal degeneration in peripheral nerve, and there is no upregulation 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.

Key words: chemotherapy neuropathy, carnitine, olesoxime, mitotoxicity, sensory neuropathy.

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, 2011; Quasthoff and Hartung, 2002; Windebank and Grisold, 2008).

The anti-cancer mechanism of action of the platinum-complex agents is caused by 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 because of 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

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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, von Frey hair.

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concurrent treatment with other neurotoxic chemotherapeutics, prior radiation therapy, comorbidities 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, 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 because of an effect of the oxalate salt on axonal sodium channels (Adelsberger 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.

MATERIALS AND METHODS

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. The number of animals used was limited to that which was necessary for proper statistical analysis. 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.

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).

Animals

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

Oxaliplatin administration

A stock solution of oxaliplatin (Sanofi-Aventis, Laval, QC, Canada; 5 mg/ml) was diluted to 2 mg/ml with 5% dextrose in distilled water, and injected i.p. 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.

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), that is, 3 and 31 days after the last injection of oxaliplatin. After an overdose of sodium pentobarbital (150 mg/kg, i.p.), urine was collected through bladder puncture, and blood was obtained through cardiac puncture. Blood was collected in heparinized tubes, and plasma was obtained through centrifugation. The following were measured: blood urea nitrogen (BUN; Quantichrom Urea Assay Kit; BioAssay Systems, Hayward, CO, USA); creatinine in plasma and urine (Enzymatic Creatinine Test Kit; Diazyme Laboratories, Poway, CA, USA); *N*-acetyl-β-D-glucosaminidase level in urine (NAG; Diazyme laboratories); and protein level in urine (Bio-Rad Dye Reagent; Bio-Rad Laboratories, Hercules, CA, USA).

Behavioral testing

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

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 with 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 super-normal pain response to a normally noxious stimulus). Withdrawal responses were counted and expressed as an overall percentage response.

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 six scores for each rat, the minimum score being 0 and the maximum possible score being 18.

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).

Axon counts

The saphenous nerve at mid-thigh level from oxaliplatin-treated and vehicle-treated rats (n=4/group) sacrificed at the time of