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Comparison of Oxaliplatin- and Cisplatin-induced Painful Peripheral Neuropathy in the Rat

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Abstract

While platinum-based cancer chemotherapies produce painful peripheral neuropathy as dose-limiting side effects, there are important differences in the pain syndromes produced by members of this class of drugs. In the rat Cisplatin-induced hyperalgesia has latency to onset of 24–48 hrs, is maximal by 72–96 hrs and is attenuated by inhibitors of caspase signaling, but not by inhibitors of the mitochondrial electron transport chain (mETC) and anti-oxidants. In contrast, Oxaliplatin-induced mechanical hyperalgesia is already present by 5 min, peaks by 20 min. While Oxaliplatin hyperalgesia persists for weeks, starting around day 10–15 its severity decreases to a lower 2nd plateau level. The rapid onset 1st plateau in Oxaliplatin-induced hyperalgesia was characterized by prominent cold allodynia and in contrast to Cisplatin was attenuated by inhibitors of the mETC, and anti-oxidants but not inhibitors of caspase signaling. However tested later, during the 2nd plateau was characterized by less intense hyperalgesia, no cold allodynia and was attenuated by inhibitors of caspase signaling, as well as by inhibitors of the mETC and by anti-oxidants.

Keywords

Cancer; Cisplatin; Oxaliplatin; Neuropathy

While platinum-based chemotherapies (e.g., Cisplatin and Carboplatin) are a mainstay for the treatment of solid tumors, especially colorectal, but also ovarian, testicular, bladder and lung cancer 29,48, their clinical use is severely curtailed by dose-limiting renal-, oto- and neurotoxicity 7,28,41. The neurotoxicity induced by this class of anti-cancer drugs is characterized by a dose-dependent painful sensory neuropathy presenting with symptoms in the distal extremities 17,39. And, while the more recent development of a third generation platin, Oxaliplatin, has provided dramatic attenuation of renal- and oto-toxicity 20,21,37, rather than having less neurotoxicity it was found to have an additional, unique, neurotoxic effect, a relatively rapid onset painful peripheral neuropathy that is exacerbated by exposure to cold 11,17,34,36. In at least 90% of patients receiving Oxaliplatin, they develop symptoms during or soon after its administration, in some patients in association with the first dose 16. Since there are currently no therapies that either cure or prevent platinum-based chemotherapy-induced painful peripheral neuropathy, there is an urgent, and with the newer members of this class of drugs growing need to better understand the underlying pathophysiology of these pain syndromes, to assist in the development of more effective therapies.

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In a previous study, we found that Oxaliplatin-induced hyperalgesia has two components, a higher level plateau hyperalgesia during the first week and a lower level plateau starting approximately 10 days after Oxaliplatin administration, from which the animals did not fully recover even after three weeks²⁴. In the present study we have tested the hypothesis that the two plateaus in Oxaliplatin-induced hyperalgesia reflect different mechanisms and that the mechanisms implicated in the second plateau of Oxaliplatin hyperalgesia have similarities to both the hyperalgesia during the first plateau as well as that observed in Cisplatin hyperalgesia.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on 220–250 g adult male Sprague–Dawley rats (Charles River, Hollister, CA). Animals were housed 3 per cage in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 A.M.). Food and water were available *ad libitum*. National Institutes of Health guidelines for care and use of animals were followed, and the protocol was approved by the University of California, San Francisco Committee on Animal Research. All efforts were made to minimize the number of animals used and their suffering.

Behavioral testing

Our previous studies²⁴ indicated a long time course (>20 days) for Oxaliplatin-induced hyperalgesia, which persisted even after the 20th day. Therefore, we measured the paw withdrawal thresholds of Oxaliplatin treated rats for another 20 days (total of ~6 weeks). A difference in the intensity of hyperalgesia was found between the earlier (days ~1–7) and the later (days ~10–30) times. Therefore, we performed further testing for each of these two plateaus in Oxaliplatin hyperalgesia. The first plateau was tested on days 1–5 and the second on days 20–25, after Oxaliplatin administration. Since Cisplatin hyperalgesia does not have a rapid onset and is shorter lived (<10 days), all studies (except the onset and time course) involving Cisplatin were done between days 3–5 post Cisplatin administration, when Cisplatin hyperalgesia is maximal.

Mechanical nociceptive threshold testing

Randall-Selitto paw-withdrawal test—Behavioral experiments were done in a quiet, temperature (20–22°C)-controlled room between 10.00 and 16.00 h. Mechanical hyperalgesia was assessed by measuring the paw-withdrawal thresholds in rats using a Basile Algesymeter[®] (Stoelting, Chicago, IL) as previously described.^{3,5} Briefly, rats were allowed to crawl into a Perspex[®] cylindrical restrainer with slanted vents on the side for the hind legs to extend freely, and acclimatized for 10–15 min, after which the hind paws were exposed to the test stimulus. The nociceptive threshold was defined as the force in grams at which the rat withdrew its paw. Each paw was treated as an independent measure and each experiment was performed on a separate group of rats. Except where stated, rats were treated with only one agonist and/or antagonist, injected intradermally on the dorsum of the hind paw. Paw-withdrawal thresholds were determined before and after drug administration. The paw withdrawal thresholds, prior to the drug administration served as its own control except in the time course study, where the vehicle treated rat served as the control. For multiple studies as in the case of mitochondrial electron transport inhibitors, the basal thresholds of all the controls and that of the oxaliplatin- or Cisplatin-treated rats are pooled. Nociceptive thresholds were determined at 5 min intervals and the mean of three readings was defined as the nociceptive threshold²⁵. The effect of test agents is expressed as percentage decrease from their pre-injection paw-withdrawal threshold (mean $\pm$ SEM).

von Frey hair (VFH) stimulation test for mechanical allodynia—To study allodynic effects of Oxaliplatin and Cisplatin, animals were placed on a mesh floor and covered by an inverted transparent plastic box (18 × 8 × 8 cm, L × W × H); open at the bottom so that a calibrated VFH monofilament (75.8 mN, Ainsworth, London, U.K.) could be applied to the plantar skin of the hind paw. This force was selected based on the results of our previous studies 6,25. The VFH was tested by inserting it from below through the mesh floor and applying it to the heel until the VFH filament just bent 32. A trial consisted of eight repetitive VFH applications (at a frequency of one per 1–1.5 s). Five trials were performed on each hind paw with a 3 min inter-trial interval. Data is reported as foot withdrawal frequency [i.e. (number of foot withdrawals/5) × 100]. Measurements taken from normal rats prior to the treatment served as the control.

Heat stimulation—We also studied the effect of Oxaliplatin and Cisplatin on the response to thermal stimuli, using the radiant heat method described by Hargreaves and colleagues 22. Each rat was placed on a glass platform, under an inverted clear acrylic box (18 × 8 × 8 cm) open at the bottom. After 30–60 min of habituation to the test apparatus, a 50 W radiant heat stimulus was projected through an oval-shaped aperture (5 × 10 mm) onto the heel of the hind paw. A photocell attached to a feedback circuit turned off the light beam stimulus when the paw was moved, recording the latency of foot withdrawal to the nearest 0.1 s. The intensity of radiant heat directed onto the hind paw was adjusted in pilot experiments, to give a response latency of approximately 10–15 s in control rats. Three measurements were taken on each hind paw with 5 min intervals between stimuli and the three measurements averaged. Measurements taken from normal rats prior to any treatment served as the control.

Cold stimulation—Cold allodynia induced by Oxaliplatin and Cisplatin was assessed by the tail immersion test in a water bath maintained at 10°C, with a cut-off time of 15 s. The time taken by the rat to withdraw its tail from the cold water was the nociceptive measure. Three measurements were taken at 5 min intervals and the three measurements averaged. Measurements taken from normal rats prior to any treatment served as the control.

Chemicals used in the study

Oxaliplatin, Cisplatin, acetyl-L-carnitine (antioxidant), α -lipoic acid (antioxidant), vitamin C (antioxidant), Rotenone (mETC 1 inhibitor), 3-NP (mETC 2 inhibitor), antimycin (mETC 3 inhibitor), NaCN (mETC 4 inhibitor), and oligomycin (mETC5 inhibitor) were purchased from Sigma (St Louis, MO). ZVAD-FMK (nonspecific caspase inhibitor) was purchased from EMD Bioscience (San Diego, CA). Intradermal (i.d.) injections were done through a beveled 30-gauge hypodermic needle, purchased from Sherwood Medical Company (St Louis, MO), attached by a piece of PE-10 polyethylene tubing to a 10 μ l microsyringe (Hamilton, Reno, NV).

Administration of test agents

Oxaliplatin and Cisplatin (both 2 mg/kg) were administered intravenously (i.v.), *via* a tail vein, all the other chemicals employed in the study were administered into the hindpaw via the intradermal (i.d.) route. Intravenous administration of drugs was followed by a bolus injection of an equal volume of saline, prior to removal of the injection needle. All agents employed in the study were dissolved in normal saline and the volume adjusted to 1 ml/kg for i.v. and 5 μ l/paw for i.d. administration. All intradermal agents except acetyl-L-carnitine, α -lipoic acid and vitamin C (each 5 μ g/paw) were administered at a dose of 1 μ g/paw, at the site of nociceptive testing. Dose selection of each agent was based on the results of our previous studies.^{4,24–26} The paw-withdrawal thresholds were determined prior to and 30 min after i.d. injections of drugs and every test was evaluated during the 1st and 2nd plateau of Oxaliplatin

hyperalgesia and between days 3–5 after Cisplatin administration. The effect of each chemical was determined on different groups of rats.

Data analysis

Group data are presented as mean $\pm$ S.E.M. and analyzed statistically using a two-tailed paired (when the same rats were used as their own control) or unpaired (when control and test groups were different) Students “t” test. Time course data for Oxaliplatin and Cisplatin are analyzed using one way ANOVA followed by Tukey’s post hoc test. The level for statistical significance was set at a *P*-value of <0.05 .

RESULTS

Intravenous Oxaliplatin and Cisplatin induce mechanical hyperalgesia

Intravenous administration of a single dose of Oxaliplatin or Cisplatin (both 2 mg/kg) produced significant (20–35%) reduction in mechanical paw-withdrawal threshold (Fig. 1B, *n* = 6/group). Cisplatin showed a delayed (1–2 days) onset compared to Oxaliplatin (30 min, Fig. 1A, 6/group) with both reaching a similar peak level (20–35% decrease in mechanical nociceptive threshold). As we have shown previously [24], Oxaliplatin hyperalgesia demonstrated two plateaus over time. The first plateau lasted approximately 1 week, and the second, a sustained lower level of hyperalgesia plateau occurred starting around 10 days post-Oxaliplatin administration and lasted more than 6 weeks. While similar in peak magnitude, Cisplatin hyperalgesia was of somewhat shorter duration (< 2 weeks, Fig. 1B).

Oxaliplatin and Cisplatin induce mechanical allodynia

VFH stimulation conducted on days 3–5 following Cisplatin, and days 3–5 (1st plateau) and days 20–25 (2nd plateau) after Oxaliplatin administration (2 mg/kg $\times$ 1, i.v.), demonstrated a significant increase in paw withdrawal frequency (Fig. 2A, *n* = 6/group, *p* < 0.001 for all), mechanical allodynia.

Oxaliplatin and Cisplatin induce heat allodynia

On exposure to the noxious radiant heat stimulus (Hargreaves method), there was a significant reduction in paw withdrawal latency in Oxaliplatin (1st and 2nd plateau) and Cisplatin-treated rats (Fig. 2B, *n* = 6/group, *p* < 0.001 for all).

Oxaliplatin induces cold allodynia during the 1st plateau

Oxaliplatin significantly reduced the response latency to cold (10°C tail immersion), starting a few hours post Oxaliplatin (Fig. 2C, *n* = 6, *p* < 0.001), that only lasted a short period (≤ 3 days). Unlike mechanical hyperalgesia, Oxaliplatin during the 2nd plateau and Cisplatin do not demonstrate cold allodynia (Fig. 2C, *n* = 6/group, both *p* > 0.05).

Second messenger inhibitors differ in their effects on the 1st versus the 2nd plateau of Oxaliplatin-induced mechanical hyperalgesia

Inhibitors of two mitochondrial electron transport chain (mETC) complexes, I (Rotenone) and III (antimycin), inhibited Oxaliplatin hyperalgesia during both the 1st and 2nd plateau. Inhibitors of the other three complexes, II (3-NP), IV (NaCN) and V (oligomycin), did not affect Oxaliplatin hyperalgesia during either plateau (Fig. 3A & B). Similarly, three different antioxidants (acetyl-L-carnitine, α -lipoic acid, and vitamin C) inhibited Oxaliplatin hyperalgesia during both plateaus (Fig. 3A & B). Finally, the non-selective caspase inhibitor (ZVAD-FMK) markedly inhibited the 2nd but not the 1st plateau in Oxaliplatin hyperalgesia (Fig. 3A & B).

Second messenger inhibitor effects for Cisplatin-induced mechanical hyperalgesia differ from Oxaliplatin hyperalgesia

Inhibitors of the mitochondrial electron transport chain complexes (I-V) and all antioxidants failed to inhibit Cisplatin hyperalgesia (Fig. 3C). However, the non-selective caspase inhibitor did inhibit Cisplatin hyperalgesia (Fig. 3C).

Discussion

While the mechanism by which many chemotherapies produce their tumoricidal effects have been established, the critically important question of whether the same mechanism is also responsible for their dose-limiting side effects remains largely unknown, especially as it relates to their effects on the peripheral nervous system. Thus, while it is generally held that the therapeutic effects of platinum-based anticancer drugs are mediated by DNA alkylation 13, 42,43, the mechanism by which they produce painful peripheral neuropathy, a major dose-limiting side effect for this class of therapeutic agents is not well understood. That the pain syndromes produced by different platinum-based chemotherapies differ so markedly suggests they may, in fact, be mediated by mechanisms unrelated to their tumoricidal action. A dramatic example of this is a unique form of painful peripheral neuropathy produced only by Oxaliplatin, characterized by a rapid onset and profound cold allodynia, a pain syndrome not produced by any of the other members of the platinum-based chemotherapies 11,14 or for that matter by other classes of cancer chemotherapies. These early symptoms produced by Oxaliplatin often disappear within a few days to a couple of weeks 11,14, reappearing with repeat cycles of chemotherapy administration. In addition to these acute symptoms, patients also develop a well-described later-onset painful peripheral neuropathy that is thought to be similar to that produced by other members of the platinum-based cancer chemotherapies 12,20. Treatment with many cancer chemotherapies is limited by their dose-related peripheral nervous system toxicity, a small-fiber painful peripheral neuropathy 1,46,47. It is critical to better understand the underlying mechanisms, to allow identification of therapeutic targets, especially if these drugs produce their neurotoxic effects by mechanisms different from those by which they kill tumor cells. In the present study we have characterized the pain syndromes produced by two commonly used members of the platinum-based cancer chemotherapies, Cisplatin, a representative drug in this class, which has been in clinical use for 4 decades, and Oxaliplatin, a diaminocyclohexane (DACH) platinum, the only third generation member of this class of drugs in clinical use. We also evaluated for differences in the relative contribution of mitochondrial mechanisms underlying the mechanical hypersensitivity produced by these two chemotherapeutic agents.

While Oxaliplatin and Cisplatin both induce a painful distal sensory neuropathy, only Oxaliplatin induces a pain syndrome with both prominent cold allodynia and rapid onset of symptoms. In the present study we found that mechanical hypersensitivity induced by Oxaliplatin has a rapid onset and cold allodynia, while that induced by Cisplatin is delayed in onset, reaching peak intensity closer to a week after administration. However, Oxaliplatin-treated rats progress over time to a syndrome with similarities to that produced by Cisplatin as it is characterized by delayed appearance, without prominent cold allodynia. It is antagonized by a second messenger inhibitor that also attenuates Cisplatin-induced painful peripheral neuropathy but not those that inhibit the rapid onset 1st plateau in Oxaliplatin-induced hyperalgesia. Thus, our study establishes models that distinguish between neuropathic pain syndromes produced by different members of a single chemical class of anticancer drugs, that are thought to produce their tumoricidal actions by the same mechanism (i.e., alkylating DNA 13,42,43).

For the acute pain syndrome observed in patients receiving Oxaliplatin, the mechanism has yet to be established. While it has been suggested to be mediated by direct effects on sodium

channel function,^{2,35} this may actually be an indirect action since the onset of symptoms is often delayed for hours.

Based on an extensive literature demonstrating effects of platinum-based cancer chemotherapies on mitochondrial function ^{18,19,23,31,38} and the demonstrated role of mitochondria in models of other forms of painful peripheral neuropathy ^{15,27} as well as in patients with painful peripheral neuropathy, ⁴⁰ we tested the hypothesis that these mitochondrial functions contribute to platinum-based chemotherapy-induced painful peripheral neuropathy.

Since increased reactive oxygen species (ROS) are the main mitochondrial mechanisms implicated, we evaluated for a contribution of mETCs, which drive ROS production in mitochondria. We found that oxaliplatin-induced mechanical hyperalgesia during both the 1st and 2nd plateau were antagonized by mETC inhibitors and by antioxidants. Importantly, inhibitors of mETC I and III, mETCs implicated in ROS production, ^{9,33} but not mETCs II, IV and V, attenuated Oxaliplatin hyperalgesia. Taken together, these data support a role for mETC driven ROS production in Oxaliplatin-induced painful peripheral neuropathy. In contrast to the pronociceptive effect of oxaliplatin, that of the closely related chemotherapeutic agent, Cisplatin, is not antagonized by either mETC inhibitors or antioxidants.

The finding of a role of caspase signaling in the 2nd plateau of Oxaliplatin hyperalgesia, as well as in Cisplatin hyperalgesia, suggests a more delayed activation of this mechanism. While this might be due to mitochondrial injury by ROS in Oxaliplatin treated rats, this mechanism seems less likely to explain the role of caspase signaling in Cisplatin hyperalgesia. Thus, while in the absence of a full dose-dependence study for each inhibitor, our results do not fully exclude a contribution of ROS in Cisplatin hyperalgesia, it would still account for only a small amount of the hyperalgesia induced by Cisplatin.

Pain is an early manifestation of many forms of peripheral neuropathies including those induced by cancer chemotherapy. Many of these neuropathies ultimately progress to sensory neuron loss. In addition, these neuropathies may experience an intermediate phase in which patients experience both pain and selective attenuation of some modalities demonstrated on quantitative sensory testing ^{8,30}. These findings raise the intriguing possibility that the mechanisms underlying cancer therapy-induced painful neuropathy may also be involved in the subsequent injury and ultimately the death of sensory neurons. This suggestion is also supported by the observation that the acute hyperexcitability produced by Oxaliplatin is greater in patients early in their course of treatment. Thus, a recent study of neuropathic pain in patients receiving Oxaliplatin found that patients with peripheral neuropathy who did not report having pain had received twice as many cycles of chemotherapy, compatible with them having already progressed to the stage of fiber death ⁸. These findings support the suggestion that there is a temporal association between acute and chronic forms of peripheral neuropathy ³⁴. Furthermore, it has been suggested that altered sodium channel function, implicated in the 1st plateau of Oxaliplatin neuropathy ²⁴ is involved in axonal degeneration as well as acute hyperexcitability ³⁴. This literature ³⁴ as well as our data supports the suggestion that pain can be an early manifestation of a process that may ultimately lead to cell death. Thus, increasing our understanding of the mechanisms mediating pain in neurotoxic peripheral neuropathies might also guide the development of neuroprotective treatments.

Unfortunately, no currently available therapies reliably prevent or treat chemotherapy-induced painful peripheral neuropathy. If it were possible to establish the mechanism of this form of neurotoxicity, chemotherapy-induced small-fiber painful peripheral neuropathy, it would provide an important advance toward the development of more effective therapies. As well, it will be important to determine the relationship between the two types of Oxaliplatin-induced

painful peripheral neuropathy and between them and Cisplatin-induced neuropathy; for example, does the 1st plateau of Oxaliplatin neuropathy contribute to induction of the 2nd plateau, and does one plateau or the other better predict subsequent cell death. Finally, clinical trials for the treatment of neuropathic pain have been based, in large part, on a very limited number of neuropathic pain syndromes, mainly post-herpetic neuralgia and diabetic neuropathy. However, growing evidence suggests that the mechanisms underlying pain in various forms of peripheral neuropathy may differ dramatically 4. This is true even for agents from the same drug class (e.g., Oxaliplatin and Cisplatin). Thus, these observations may help to explain why our current treatments 10,44,45,49 for painful peripheral neuropathies have not been more effective.

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Figure 1.

A. Time course of Oxaliplatin (0.5 mg/kg, i.v. reported from 24 to show the two plateau of Oxaliplatin hyperalgesia, and 2 mg/kg, i.v., $n = 12/\text{group}$) and Cisplatin (2 mg/kg/i.v. $n = 6/\text{group}$) induced mechanical hyperalgesia. Oxaliplatin (2 mg/kg, i.v.) induced hyperalgesia lasted over 6 weeks and on the 42nd day there still was significant ($p < 0.01$) hyperalgesia, while Cisplatin hyperalgesia lasted less than 2 weeks. Also there was a significant difference in the intensity of Oxaliplatin (2 mg/kg, i.v.) hyperalgesia between day 5 and day 20 ($p < 0.01$). **B.** Onset of mechanical hyperalgesia induced by Oxaliplatin and Cisplatin (both 2 mg/kg/i.v. $n = 6/\text{group}$). Oxaliplatin induced hyperalgesia has markedly faster onset than Cisplatin hyperalgesia.

Figure 2.

A. Mechanical allodynia induced by Oxaliplatin (1st and 2nd plateau) and Cisplatin (n = 6/group). **B.** Effect of Oxaliplatin (1st and 2nd plateau) and Cisplatin (n = 6/group) on response to noxious heat, measured by Hargreaves test, and **C.** Effect of Oxaliplatin (1st and 2nd plateau) and Cisplatin (n = 6/group), on response to noxious cold (10°C). The level of significance is denoted by (*) where $p < 0.05 = *$, $p < 0.01 = **$ and $p < 0.001 = ***$. OXP = Oxaliplatin, CISP = Cisplatin.

Figure 3.

Effect of mitochondrial electron transport complex (mETC) inhibitors rotenone, 3-NP, antimycin, sodium cyanide and oligomycin, antioxidants acetyl-L-carnitine (ALC), α -lipoic acid (α -lipo) and vitamin C, and a caspase inhibitor (ZVAD-FMK) on the **A.** acute, and **B.** chronic effect of Oxaliplatin-induced mechanical hyperalgesia, and **C.** on Cisplatin-induced mechanical hyperalgesia (n=6/group). All inhibitors were administered to different groups of Oxaliplatin and Cisplatin treated rats.