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Persistent Hyperalgesia in the Cisplatin-Treated Mouse as Defined by Threshold Measures, the Conditioned Place Preference Paradigm and Changes in Dorsal Root Ganglia Activated Transcription Factor 3: The Effects of Gabapentin, Ketorolac and Etanercept

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Abstract

Background—Painful neuropathy is a dose-limiting side effect in cancer chemotherapy. To characterize this phenomenon, we examined pain behavior and analgesic actions in a mouse model of cisplatin polyneuropathy.

Methods—Male C57BL/6 mice received intraperitoneal (i.p.) cisplatin or saline (2.3 mg/kg/day) every other day 6 times over 2 weeks for a total dose of 13.8 mg/kg. Thermal escape latencies, mechanical allodynia using von Frey hairs and observation of behavior/morbidity and body weights were assessed. After onset of allodynia, we examined the actions of i.p. gabapentin (100 mg/kg), etanercept (20, 40 mg/kg), ketorolac (15 mg/kg), and morphine (1, 3 and 10 mg/kg). Additionally, using the conditioned place preference (CPP) paradigm, we examined the effects of gabapentin and ketorolac on the presumed pain state initiated by cisplatin. Additionally, we examined the spinal cord and dorsal root ganglia (DRG) of cisplatin-treated mice.

Results—Cisplatin, but not saline treatment, produced persistent hind paw tactile allodynia which persisted 46 days with no effect on thermal escape. Gabapentin and morphine, but neither etanercept nor ketorolac, produced a complete but transient (2 h) reversal of the allodynia. Etanercept (40 mg/kg) pretreatment resulted in a delay in onset of mechanical allodynia. Using CPP, gabapentin, but not ketorolac, in cisplatin animals resulted in a significant preference for the drug-associated treatment compartment. There was no place preference in noncisplatin-treated (nonallodynic) mice after gabapentin injection. Immunohistochemistry in cisplatin-treated mice showed no change in GFAP (astrocyte) or Iba1 (microglia) activation states, but a significant increase in Activated Transcription Factor 3 (ATF3) was observed in the DRG.

Conclusions—Cisplatin-treated mice display allodynia and an activation of DRG ATF3 which is paralleled by its effects on behavior in the CPP system, wherein gabapentin, but not ketorolac, in the presence of the cisplatin polyneuropathy, is positively rewarding, confirming that this neuropathy is an aversive (painful) state that is ameliorated by gabapentin.

Introduction

Chemotherapy-evoked neuropathic pain is one of the major side effects of cancer chemotherapy.[1] The associated pain state can be severe enough to lead to termination of treatment.[2] Cisplatin (Cis-diamminedichloroplatinum (II)) is a chemotherapy drug used to treat a variety of cancers. The platinum complex drugs which are widely used antineoplastic agents serve to crosslink DNA leading to apoptosis.[2,3] Common to many antineoplastic drugs, the therapeutic protocols for cisplatin used to treat cancers will typically yield sensory neuropathies characterized by pain and paresthesias in distal extremities occurring over intervals of days to weeks.[4]

Although the exact etiology of chemotherapy-induced neuropathy is unknown,[5] advances in understanding this phenomena and the development of potentially ameliorating pain therapies has resulted in the development of robust preclinical models. In the mouse, platin agents such as cisplatin and oxalipatin produce a tactile allodynia as measured by von Frey hairs and thermal hyperalgesia when used at total doses up to 15 mg/kg with treatment delivered over 20 days.[6,7] Several studies have suggested that the pathology resembles that of neuropathic rather than an inflammatory condition. One example of this has been changes in the dorsal root ganglion (DRG) expression of activating transcription factor 3 (ATF3), a reported marker of nerve injury in paclitaxel- and cisplatin-treated but not vincristine-treated animals.[8–10]

An important element of this work on chemotherapeutic models has been that all of the paradigms have used threshold measurement. As noted in humans, aside from changes in mechanical sensitivity, patients report continuing dysaesthesia. Recent work has indicated

that aside from threshold measurements the “painful” nature of the treatment may be addressed by the conditioned place preference paradigm (CPP).[11–13] In this paradigm, if the animal i) has pain and ii) if a drug ameliorates that pain, then that drug will have a positive reinforcing property such that if the drug is given in a particular environment, the animal will come to demonstrate a preference for that environment, e.g., a CPP. Conversely, such drugs would not have rewarding properties in the absence of the hypothesized pain state. This phenomena has hitherto not been examined in a chemotherapeutic polyneuropathy model.

Accordingly, in the present studies, we sought to determine: i) the effects of cisplatin treatment on thermal and mechanical thresholds, ii) the effects of this treatment on DRG ATF3 and spinal glial activation, iii) the effects of drugs with antihyperalgesic actions after tissue and/or nerve injury (ketorolac, gabapentin, morphine, and etanercept; because these drugs are nonsteroidal antiinflammatory drugs (NSAIDs), anticonvulsants, opioids, and tumor necrosis factor (TNF)-α antagonist used in pain control, respectively) on the cisplatin-induced hyperalgesia, and iv) whether the cisplatin-treated animals would display a preference for a chamber in which it had received a drug treatment that relieved its allodynia. The present results support the assertion that the cisplatin model is indeed representative of a hyperalgesic state and that state responds to the antihyperalgesic drug gabapentin, but not the NSAID, ketorolac.

Methods

This study was approved by the Institutional Animal Care and Use Committee at the University of California, San Diego. Male C57BL/6 mice (25–30 g) were used in these studies. Food and water were provided freely. Up to 4 mice were housed in plastic cages with soft bedding, and were maintained on a 12:12-hr light-dark cycle.

Cisplatin treatment

Mice received intraperitoneal (i.p.) injections of either cisplatin (Spectrum Chemical MFG., Gardena, CA, USA) (2.3 mg/kg/day; $n = 6$, respectively) or saline every other day, 6 times over 2 weeks for a total dose of 13.8 mg/kg. Between cisplatin injection days, lactated Ringer’s solution (0.25 ml) was injected to maintain hydration and to protect the kidney and liver. This treatment/dosing paradigm was selected on the basis of preliminary studies demonstrating that this treatment regimen resulted in robust allodynia with acceptable body weight loss. On day 15, animals that showed a foot withdrawal response to von Frey filaments with an applied bending force of 0.6 g or less were considered neuropathic and used in subsequent studies.

Drug Administration

Neuropathic mice, on or around day 15, were randomly assigned to receive i.p. gabapentin (100 mg/kg), etanercept (20, 40 mg/kg), ketorolac (15 mg/kg), morphine (1, 3 and 10 mg/kg), or saline (vehicle). In another group of animals, we undertook a pretreatment regimen of etanercept (40 mg/kg) or saline delivered just before the first cisplatin injection ($n = 4$–6 per group).

Behavioral Tests

All behavioral tests were conducted at fixed times (9:00 a.m.–5:00 p.m.). The behavioral test for mechanical allodynia was conducted just before the daily injection during the course of cisplatin treatment. For the drug treatment studies, mechanical allodynia was determined preadministration and at 15, 30, 60, 90, 120, 180, 240, and 1440 min after i.p. drug administration. The thresholds for mechanical allodynia were measured with a series of von

Frey filaments (Semmes Weinstein von Frey aesthesiometer, Stoelting Co., Wood Dale, IL, USA), ranging from 2.44 to 4.31 (0.03–2.00 g) using the up-down method.[14] We assessed thermal escape responses using a Hargreaves-type testing device (UARDG, Department of Anesthesiology, University of California, San Diego) as described previously.[15] All results were reported as the mean value of readings from each of the hindpaws.

Conditioned Place Preference Tests

Using a modification of the method previously described,[12] we tested CPP for gabapentin and ketorolac in cisplatin (allodynic) and vehicle control (nonallodynic) animals. Using a 3-compartment (A, B, C) box (each compartment measuring 90 × 90 × 165 cm) with the B compartment (center) separated from each of the adjacent compartments by a divider with an entryway. The A and C compartments had different patterned walls and texturally distinct floors. The obscuration by the animal of the light path of three red LED lights in Chambers A and C were used to measure the time spent in each chamber. The testing paradigm was as follows. On days 1 and 2, animals were allowed to explore freely for 30 min and the time spent in each chamber was determined. On days 3 and 4, in the morning, the animal was placed for 30 min in one closed chamber, immediately after receiving vehicle. In the afternoon (4 hrs later), the mouse was placed for 30 min in the other chamber after receiving the test drug. On day 5, the animal was placed in the start chamber (B) and allowed free access for 30 min to either chamber. The time spent in the two chambers was recorded. Chamber pairings were counterbalanced. To define drug effect, the mean of the time spent in the drug-associated chamber during adaptation (days 1 and 2) was subtracted from the time spent in that chamber on the test day (day 5).

Immunohistochemistry

On day 30, The lumbar spinal cords (L4–L6) and DRGs were harvested as described previously.[16] Spinal cord sections (30 μm) were incubated with anti-GFAP antibody (1:10000 Chemicon, Temecula, CA) or anti-Iba1 antibody (1:1000 Wako, Richmond, VA). Binding sites were visualized with secondary antibodies conjugated with fluoro-Alexa-488 and Alexa-594 (1:1000, Molecular Probes, Eugene, OR). For DRGs (10 μm), nonspecific binding was blocked by incubation in 2% normal goat serum in phosphate-buffered saline with 0.3% Triton X-100 followed by incubation with primary ATF3 antibody (generated in rabbit, 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C under gentle agitation. Binding sites were visualized with anti-rabbit immunoglobulin (IgG) antibodies conjugated with Alexa-488 (1:500, Invitrogen). Nuclei were counterstained using Topro3 (1:500; Invitrogen). All images were captured by Leica TCS SP5 confocal imaging system and quantified using Image-Pro Plus v.5.1 software. Microglia (Iba1), astrocyte (GFAP), and ATF3 staining was quantified by measuring the total integrated intensity of pixels divided by the total number of pixels in a standardized area. Staining intensity was examined in laminae I–II of the superficial dorsal horn using a standardized box with 4–6 mice per experimental condition. Only pixels above a preset background threshold were included. An increase in the integrated intensity/pixels for Iba1 and GFAP staining was interpreted to signify microglia and astrocyte reactivity, respectively. Iba1 and GFAP data were presented as the total threshold intensity area. ATF3 data are presented as percentage change from the corresponding control group. Statistics are performed on raw data values.

Statistical Analysis

Results are expressed as mean ± standard error (SE). Statistical analysis was performed using GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA). For comparison of mechanical and thermal hyperalgesia, 2-way analysis of variance (ANOVA) was used to determine general differences, depending on the treatment group and time. This was followed by Bonferroni multiple comparisons test. For CPP tests, data were analyzed before

conditioning (mean) and after conditioning using 1-way ANOVA followed by a Newman-Keuls multiple comparison test. Group differences were analyzed using paired t-tests. Difference scores were calculated for each mouse using the formula: test time in chamber – preconditioning mean time spent in chamber. For comparison of microglia, astrocyte, and ATF3 changes, a t-test was used. A P value of < 0.05 was considered significant.

Results

Cisplatin treatment produces significant and persistent mechanical allodynia

None of the cisplatin-injected mice showed any motor dysfunction or complications. No animal required early euthanasia.

Mechanical withdrawal threshold was significantly reduced after cisplatin treatment from day 3 and through day 30 as compared to the baseline ($P < 0.0001$, Fig. 1A). There were no changes in thermal escape latency when measured on day 29 ($P > 0.05$, Fig. 1B). The cisplatin-injected mice showed a modestly retarded weight gain as compared to vehicle-treated mice during the initial dosing, but there was no statistical difference in the posttreatment phase with normal weight gain after cisplatin treatment ($P > 0.05$, Fig. 1C). All animals with demonstrated allodynia showed normal symmetrical ambulation with no loss of placing or stepping responses. Cisplatin, but not vehicle, produced significant and persistent mechanical allodynia.

Analgesic pharmacology of cisplatin evoked tactile allodynia

In mice with established allodynia (approximately 15–19 days after initiation of cisplatin treatment), gabapentin (100 mg/kg, i.p.) and morphine (10 mg/kg, but not 1 or 3 mg/kg, i.p.) treatment resulted in a reversal of the allodynia for periods of 30 through 240 min ($P < 0.0001$, Fig. 2A) and of 30 through 180 min ($P < 0.0001$, Fig. 2C) after drug injection, when compared to vehicle, respectively. In contrast, neither ketorolac (15 mg/kg, i.p.) nor etanercept (20 or 40 mg/kg, i.p.) altered the highly significant allodynia as compared to vehicle treatement ($P < 0.0001$, Fig. 2B and 3A). In separate groups, pretreatment (e.g., before the first cisplatin injection) with etanercept (40 mg/kg, i.p.) delayed the onset of allodynia through day 12 ($P < 0.0001$, Fig. 3B).

Conditioned Place Preference

Preconditioning times spent in the saline- or gabapentin- or ketorolac-paired chambers did not differ across all treatment groups ($P > 0.05$, data not shown). In initial studies, we used the paradigm of a 3-day adaptation, 1-day drug pairing and 1-day test paradigm. With the 1-day treatment schedule, gabapentin administration (100 mg/kg, i.p.) induced place preference in cisplatin-treated mice as compared to saline vehicle mice ($P < 0.05$, data not shown), but there was no significant difference from baseline scores in the gabapentin and saline groups ($P > 0.05$, data not shown). We then used a 2-day drug treatment schedule. In the 2-day drug treatment paradigm, gabapentin administration (100 mg/kg, i.p.) induced a significant place preference in cisplatin-treated mice ($P < 0.05$ vs. preconditioning, vs. saline, and vs. ketorolac). The difference from baseline scores confirmed that 2-day gabapentin treatment showed stronger positive reinforcement than the 1-day treatment in the cisplatin mice treated with gabapentin ($P < 0.05$ vs. saline, Fig. 4A). In contrast, sham (non-cisplatin) mice that were treated with gabapentin showed no difference in time spent on test day in the gabapentin- and saline-paired chambers ($P > 0.05$ vs. saline, Fig. 4B). This indicates that gabapentin with a 2-day drug pairing paradigm will initiate a significant place preference in the presence of allodynia, but in the absence of allodynia it has no ability to initiate a CPP (i.e., in the normal animal it is not positive reinforcing).

Immunohistochemistry

The threshold intensity area of both microglia (Iba1) and astrocyte (GFAP) in the spinal cords of cisplatin mice showed a modest numerical increase in cisplatin-treated mice. However, this difference was not statistically significant as compared to the control group ($P > 0.05$, Fig. 5A–F). In vehicle-treated mice, there were few ATF3 (+) cells. In contrast, cisplatin-treated mice showed significant activation of neuronal ATF3 as compared to the control group ($P < 0.05$, Fig. 6A–C).

Discussion

This study demonstrates that cisplatin initiates robust allodynia that is attenuated by gabapentin and morphine, but not by ketorolac or etanercept. Importantly, this antiallodynic effect of gabapentin paralleled the observation that in the cisplatin-treated animal, gabapentin had a positive reinforcing effect leading to a CPP.

Cisplatin dosing

In humans, cisplatin in doses of 8.1–12.2 mg/kg or 300–450 mg/m^2 body area over intervals of 3 to 4 weeks lead to robust tactile allodynia and ongoing dysesthesia.[6,17–20] We found that total doses of 13.8 mg/kg, equivalent to 41.4 mg/m^2 in mice delivered in divided doses led to allodynia after 3–5 days. In the present work, animals received i.p. lactated Ringer's solution, which reduced the toxic effects of the therapy on peripheral organ systems.[21] While higher dosing has been reported,[6,7] the present paradigm resulted in pain correlates that were accompanied by only modest weight loss, no evident morbidity or any effects upon the placing and stepping reflexes, confirming that the behavioral changes reported here were not the result of changes in motor function or from loss of response to light touch.

Cisplatin pain phenotype

The pain behavior exhibited by cisplatin mice in our study is comparable to that which has been previously reported.[6,7] Although several groups have reported thermal hyperalgesia,[6,22] we failed to see effects upon thermal thresholds, in agreement with other investigators.[23–25] An important component of this model is persistent tactile allodynia. In these cisplatin mice, such allodynia extended to at least 46 days.[6,7] This persistency is in agreement with the dysaesthesia reported in the human chemotherapy patient.[17,19]

Mechanisms underlying allodynia

As a therapeutic drug, cisplatin exerts its activity by inhibiting transcription through binding to DNA.[26] Previous work has shown that DRG neurons accumulate Pt-DNA adducts over time after cisplatin exposure.[27] This family of agents does not produce axon loss in mice.[28] In the present work, we found that glial activation typical of nerve injury was not observed. Interestingly, in the cisplatin-treated mice displaying allodynia, there was an increase in ATF3, a marker of nerve injury. Previous work has shown that ATF3 can be increased after intense peripheral stimulation.[10] The significant changes may reflect upon the nominal effects afferent integrity and perhaps a direct effect, upon the DRG. Other possibilities include the potential effects on mitochondria.[29]

Antiallodynic pharmacology

Here we show that allodynia was readily reversed by gabapentin and morphine, but not by ketorolac or etanercept. There has been no study that has described the effect of ketorolac, etanercept, and gabapentin in a cisplatin-induced neuropathic model. There are several reports regarding gabapentin in paclitaxel- and vincristine-induced neuropathic models.[30,31] Gabapentin acts by binding to the alpha2delta subunit of the calcium channel. This drug has

efficacy in allodynia, which occurs after nerve injury[32] or chronic inflammation.[16,33] Ketorolac and etanercept have been shown to have effects in models of inflammatory hyperalgesia.[16,34,35] The absence of these effects in the cisplatin model distinguishes this allodynia from models of local inflammation. Pretreatment with etanerecept was effective in delaying onset of mechanical allodynia suggesting that during the early phase of allodynia, TNF plays a mediating role. The absence of posttreatment effects with etanercept was unexpected. Previous work in neuropathic pain has shown this drug to be relatively effective in several models of established mono- and polyneuropathy such as sciatic nerve constriction injury and diabetic neuropathy.[36–38] The role of TNF in this model is thus not clear, but it does not play a sustaining role in established cisplatin-initiated allodynia. This transition to a TNF-insensitive later phase thus sets the chemotherapy model apart from other models of neuropathy.

Conditioned place preference

An important component of the present work was the examination of the effects of drugs on the cisplatin-induced state using the CPP model. While chemotherapeutics, such as cisplatin, can produce changes in mechanical and thermal threshold measures, there is controversy about the meaning of such endpoints in the preclinical evaluation of drug efficacy in persistent pain states. Of interest has been the implementation of the CPP model to define the effects of analgesic drugs in these persistent pain models.[11,12] In this paradigm, there is the assertion that i) the animal is in a state of discomfort (pain); ii) a specific drug targets that pain state and iii) the drug has no intrinsic reinforcing properties. In this model, the animal is exposed to a drug at doses that are believed be analgesic, or to a vehicle, each in a distinct environment. Later in a test period, the animal is given the choice of being in either a drug or vehicle chamber. If the animal is in discomfort and if the drug alters that discomforting state, it is presumed that the pain relief becomes a positive rewarding element and the animal will choose the environment in which it received that drug therapy. In the present work, we attempted to define the effects of gabapentin and ketorolac in establishing a place preference. Gabapentin has efficacy in a variety of nerve injury models associated with allodynia[39] and has demonstrated efficacy in cisplatin neuropathy as defined by robust effects upon the von Frey thresholds. As indicated, with two, but not a single drug pairing, the effects of gabapentin, at a dose that was observed to reverse tactile allodynia, established a robust place preference. In contrast, ketorolac had no effect. The need for two-day versus one-day drug pairing paradigm is a property consistent with the learning nature of the drug-related response. An essential control in this paradigm was that treatment of the normal animal (e.g., not displaying allodynia) with gabapentin failed to result in a place preference, indicating that gabapentin in the absence of a proposed state of facilitated pain processing had no reinforcing actions. The effects thus depended upon the pain state of the cisplatin-treated mouse. These studies were not performed with systemic morphine because it can initiate a place preference in the absence of any abnormal sensory condition (e.g., unlike gabapentin or ketorolac, it has intrinsic rewarding properties.)

Our studies validate the presence of a hyperalgesic state in cisplatin-treated mice and reveal a correlation between the pharmacology of that hyperalgesic state with persistent allodynia as defined by the CPP model. The up-regulation of ATF3 in the DRG in the absence of prominent spinal glial activation suggests a change in afferent processing resembling injury to the peripheral nerve.

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References

1. Quasthoff S, Hartung HP. Chemotherapy-induced peripheral neuropathy. J Neurol. 2002; 249:9–17. [PubMed: 11954874]
2. Wolf S, Barton D, Kottschade L, Grothey A, Loprinzi C. Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. Eur J Cancer. 2008; 44:1507–15. [PubMed: 18571399]
3. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer. 2007; 7:573–84. [PubMed: 17625587]
4. Jaggi AS, Singh N. Mechanisms in cancer-chemotherapeutic drugs-induced peripheral neuropathy. Toxicology. 2012; 291:1–9. [PubMed: 22079234]
5. Kaley TJ, Deangelis LM. Therapy of chemotherapy-induced peripheral neuropathy. Br J Haematol. 2009; 145:3–14. [PubMed: 19170681]
6. Ta LE, Low PA, Windebank AJ. Mice with cisplatin and oxaliplatin-induced painful neuropathy develop distinct early responses to thermal stimuli. Mol Pain. 2009; 5:9. [PubMed: 19245717]
7. Authier N, Gillet JP, Fialip J, Eschalier A, Coudore F. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Exp Neurol. 2003; 1:12–20. [PubMed: 12821373]
8. Jimenez-Andrade JM, Peters CM, Mejia NA, Ghilardi JR, Kuskowski MA, Mantyh PW. Sensory neurons and their supporting cells located in the trigeminal, thoracic and lumbar ganglia differentially express markers of injury following intravenous administration of paclitaxel in the rat. Neurosci Lett. 2006; 405:62–7. [PubMed: 16854522]
9. Flatters SJ, Bennett GJ. Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction. Pain. 2006; 122:245–57. [PubMed: 16530964]
10. St Germain C, Niknejad N, Ma L, Garbuio K, Hai T, Dimitroulakos J. Cisplatin induces cytotoxicity through the mitogen-activated protein kinase pathways and activating transcription factor 3. Neoplasia. 2010; 12:527–38. [PubMed: 20651982]
11. Qu C, King T, Okun A, Lai J, Fields HL, Porreca F. Lesion of the rostral anterior cingulate cortex eliminates the aversiveness of spontaneous neuropathic pain following partial or complete axotomy. Pain. 2011; 152:1641–8. [PubMed: 21474245]
12. King T, Qu C, Okun A, Mercado R, Ren J, Brion T, Lai J, Porreca F. Contribution of afferent pathways to nerve injury-induced spontaneous pain and evoked hypersensitivity. Pain. 2011; 152:1997–2005. [PubMed: 21620567]
13. Okun A, Liu P, Davis P, Ren J, Remeniuk B, Brion T, Ossipov MH, Xie J, Dussor GO, King T, Porreca F. Afferent drive elicits ongoing pain in a model of advanced osteoarthritis. Pain. 2012; 153:924–33. [PubMed: 22387095]
14. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994; 53:55–63. [PubMed: 7990513]
15. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL. Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. J Neurosci Methods. 1997; 76:183–91. [PubMed: 9350970]
16. Christianson CA, Corr M, Firestein GS, Mobargha A, Yaksh TL, Svensson CI. Characterization of the acute and persistent pain state present in K/BxN serum transfer arthritis. Pain. 2010; 151:394–403. [PubMed: 20739123]
17. Siegal T, Haim N. Cisplatin-induced peripheral neuropathy. Frequent off-therapy deterioration, demyelinating syndromes, and muscle cramps. Cancer. 1990; 66:1117–23. [PubMed: 2169332]
18. Cavaletti G, Marzorati L, Bogliun G, Colombo N, Marzola M, Pittelli MR, Tredici G. Cisplatin-induced peripheral neurotoxicity is dependent on total-dose intensity and single-dose intensity. Cancer. 1992; 69:203–7. [PubMed: 1309303]

19. Krarup-Hansen A, Helweg-Larsen S, Schmalbruch H, Rørth M, Krarup C. Neuronal involvement in cisplatin neuropathy: prospective clinical and neurophysiological studies. Brain. 2007; 130:1076–88. [PubMed: 17301082]
20. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey and man. Cancer Chemother Rep. 1966; 50:219–44. [PubMed: 4957125]
21. Maines MD. Effect of cis-platinum on heme, drug, and steroid metabolism pathways: possible involvement in nephrotoxicity and infertility. Crit Rev Toxicol. 1990; 21:1–20. [PubMed: 2264925]
22. Joseph EK, Levine JD. Comparison of oxaliplatin- and cisplatin-induced painful peripheral neuropathy in the rat. J Pain. 2009; 10:534–41. [PubMed: 19231296]
23. Hori K, Ozaki N, Suzuki S, Sugiura Y. Upregulations of P2X(3) and ASIC3 involve in hyperalgesia induced by cisplatin administration in rats. Pain. 2010; 149:393–405. [PubMed: 20378247]
24. Garcia JM, Cata JP, Dougherty PM, Smith RG. Ghrelin prevents cisplatin-induced mechanical hyperalgesia and cachexia. Endocrinology. 2008; 149:455–60. [PubMed: 17962345]
25. Cata JP, Weng HR, Lee BN, Reuben JM, Dougherty PM. Clinical and experimental findings in humans and animals with chemotherapy-induced peripheral neuropathy. Minerva Anestesiol. 2006; 72:151–69. [PubMed: 16493391]
26. Jung Y, Lippard SJ. Direct cellular responses to platinum-induced DNA damage. Chem Rev. 2007; 5:1387–407. [PubMed: 17455916]
27. Ta LE, Espeset L, Podratz J, Windebank AJ. Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology. 2006; 6:992–1002. [PubMed: 16797073]
28. Verdu E, Vilches JJ, Rodriguez FJ, Ceballos D, Valero A, Navarro X. Physiological and immunohistochemical characterization of cisplatin-induced neuropathy in mice. Muscle Nerve. 1999; 3:329–40. [PubMed: 10086893]
29. Zheng H, Xiao WH, Bennett GJ. Functional deficits in peripheral nerve mitochondria in rats with paclitaxel- and oxaliplatin-evoked painful peripheral neuropathy. Exp Neurol. 2011; 232:154–61. [PubMed: 21907196]
30. Gauchan P, Andoh T, Ikeda K, Fujita M, Sasaki A, Kato A, Kuraishi Y. Mechanical allodynia induced by paclitaxel, oxaliplatin and vincristine: different effectiveness of gabapentin and different expression of voltage-dependent calcium channel alpha(2)delta-1 subunit. Biol Pharm Bull. 2009; 32:732–4. [PubMed: 19336915]
31. Xiao W, Boroujerdi A, Bennett GJ, Luo ZD. Chemotherapy-evoked painful peripheral neuropathy: analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit. Neuroscience. 2007; 144:714–20. [PubMed: 17084535]
32. Morimoto S, Ito M, Oda S, Sugiyama A, Kuroda M, Adachi-Akahane S. Spinal mechanism underlying the antiallodynic effect of gabapentin studied in the mouse spinal nerve ligation model. J Pharmacol Sci. 2012; 118:455–66. [PubMed: 22447299]
33. Vonsy JL, Ghandehari J, Dickenson AH. Differential analgesic effects of morphine and gabapentin on behavioural measures of pain and disability in a model of osteoarthritis pain in rats. Eur J Pain. 2009; 13:786–93. [PubMed: 18955000]
34. López-Muñoz FJ, Díaz-Reval MI, Terrón JA, Déciga-Campos M. Analysis of the analgesic interactions between ketorolac and tramadol during arthritic nociception in rat. Eur J Pharmacol. 2004; 484:157–65. [PubMed: 14744599]
35. Inglis JJ, Nissim A, Lees DM, Hunt SP, Chernajovsky Y, Kidd BL. The differential contribution of tumour necrosis factor to thermal and mechanical hyperalgesia during chronic inflammation. Arthritis Res Ther. 2005; 7:R807–16. [PubMed: 15987482]
36. Sommer C, Schäfers M, Marziniak M, Toyka KV. Etanercept reduces hyperalgesia in experimental painful neuropathy. J Peripher Nerv Syst. 2001; 6:67–72. [PubMed: 11446385]
37. Zanella JM, Burright EN, Hildebrand K, Hobot C, Cox M, Christoferson L, McKay WF. Effect of etanercept, a tumor necrosis factor-alpha inhibitor, on neuropathic pain in the rat chronic constriction injury model. Spine (Phila Pa 1976). 2008; 33:227–34. [PubMed: 18303453]

38. Dogrul A, Gul H, Yesilyurt O, Ulas UH, Yildiz O. Systemic and spinal administration of etanercept, a tumor necrosis factor alpha inhibitor, blocks tactile allodynia in diabetic mice. Acta Diabetol. 2011; 48:135–42. [PubMed: 21104419]

39. Blackburn-Munro G, Erichsen HK. Antiepileptics and the treatment of neuropathic pain: evidence from animal models. Curr Pharm Des. 2005; 11:2961–76. [PubMed: 16178756]

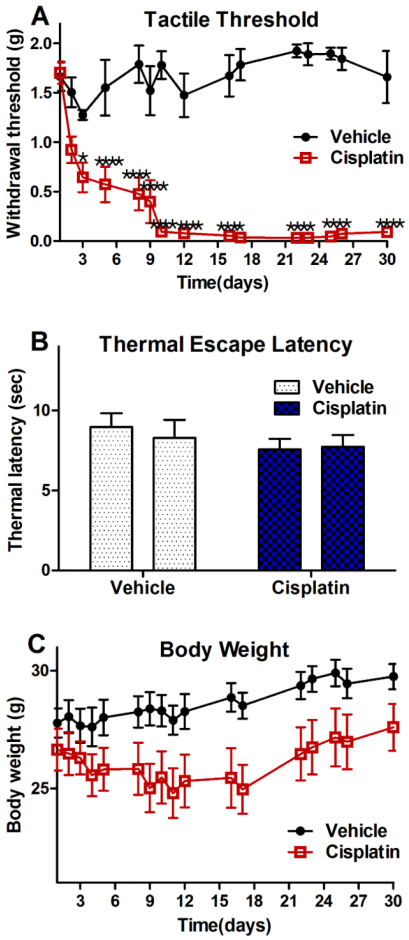

Fig. 1.

A. Figure presents the tactile threshold plotted vs. time after the initiation of cisplatin or saline treatment (six i.p. injections, one every other day). B. Thermal escape latencies measured before and at 29 days after the initiation of cisplatin or vehicle treatment. C. Changes in body weight of cisplatin-injected mice and vehicles plotted vs. time after the initiation of cisplatin or vehicle treatment. Results are presented as mean ± standard error (n = 6 in each group) ($^{*}P < .05$, $^{****}P < .0001$).

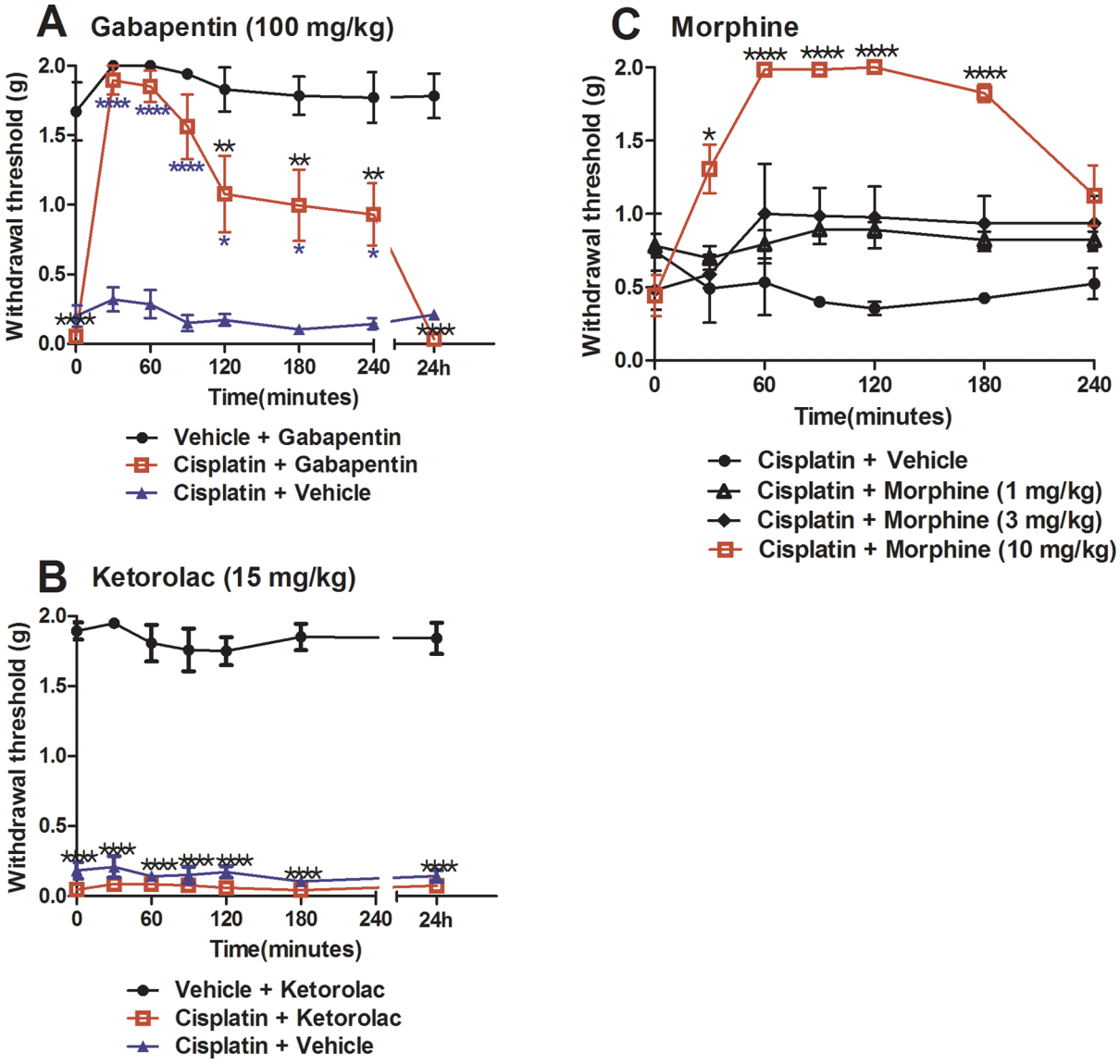

Fig. 2.

Figure plots tactile thresholds in the cisplatin-induced neuropathic mice before and after injection of gabapentin (100 mg/kg, A), ketorolac (15 mg/kg, B), and morphine (1, 3, and 10 mg/kg, C). Results are presented as mean ± standard error ($n = 4$–6 per group) ($^{*}P < .05$, $^{**}P < .01$, $^{****}P < .0001$).

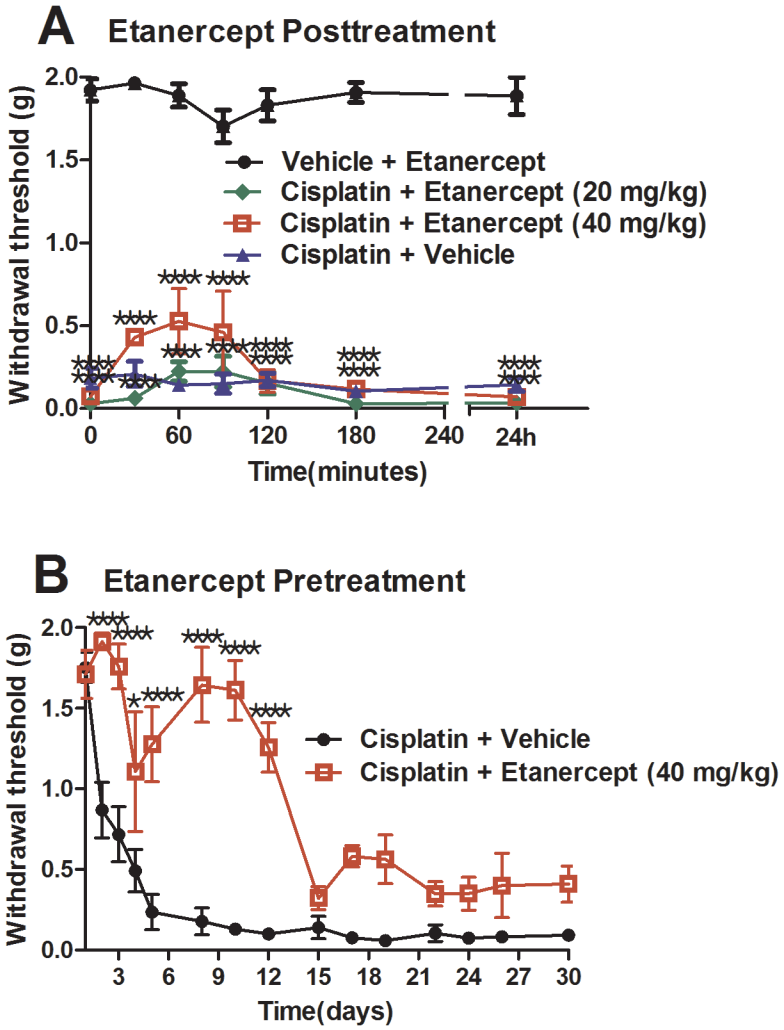

Fig. 3.

Figure plots effects of etanercept for posttreatment on tactile thresholds in the cisplatin-induced neuropathic mouse. A. Mouse received posttreatment etanercept (20 or 40 mg/kg, i.p.) or vehicle 15 days after initiation of cisplatin. B. Mouse received pretreatment with etanercept (40 mg/kg, i.p.) immediately before the initiation of cisplatin injections. Results are presented as mean ± standard error ($n = 4$–6 per group) ($^{*}P < .05$, $^{**}P < .01$, $^{****}P < .0001$).

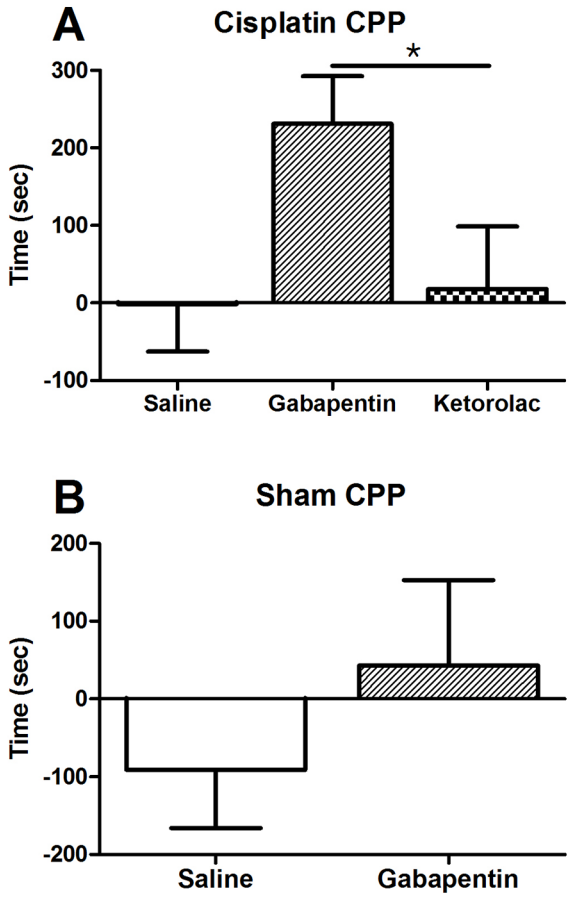

Fig. 4.

Figure presents time spent in the drug (saline-control, gabapentin or ketorolac) paired chamber in the conditioned place preference (CPP) paradigm. (A) Animals that were cisplatin-treated (allodynic) underwent CPP with gabapentin (100 mg/kg, i.p.) or ketorolac (15 mg/kg, i.p.). (B) Animals that were noncisplatin-treated (nonallodynic) underwent CPP with gabapentin (100 mg/kg, i.p.). Results are presented as mean ± standard error ($n = 5–7$ per group) ($^{*}P < .05$)

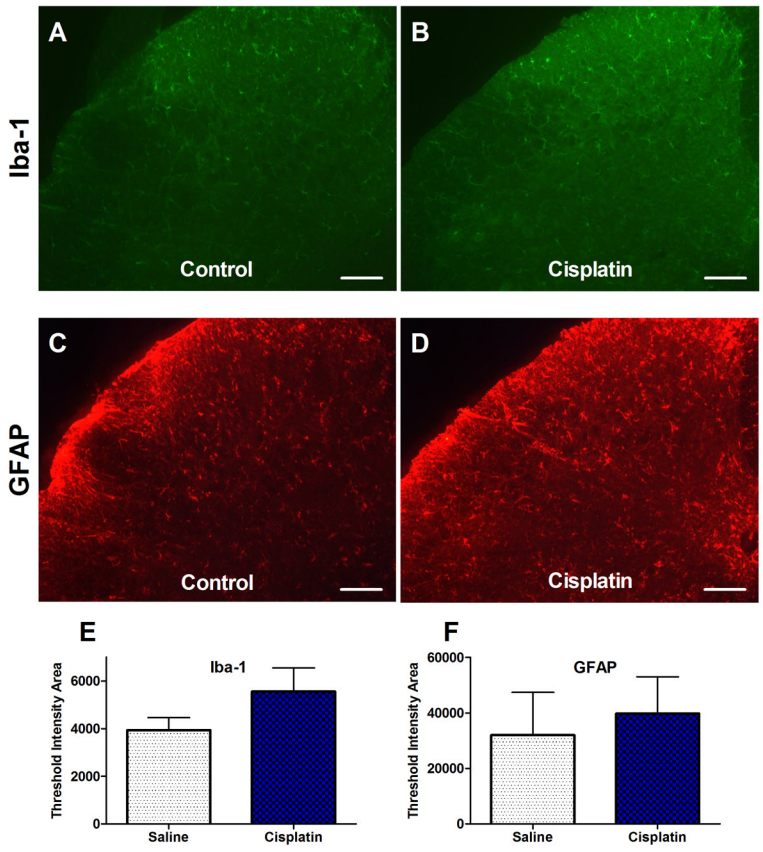

Fig. 5.

Figure presents spinal immunohistochemistry of A, B: microglia (Iba1) or C,D: astrocyte (GFAP) in spinal dorsal horn of saline (control) or cisplatin-treated mice harvested 30 days after initiation of treatment. E,F: Quantification of immune-staining intensity for Iba1 and GFAP staining in dorsal horn of cisplatin mice. As indicated intensity was numerically increased slightly, but change was not statistically significant as compared to control group (n = 6–7 per group). Scale bars represent 100 μm.

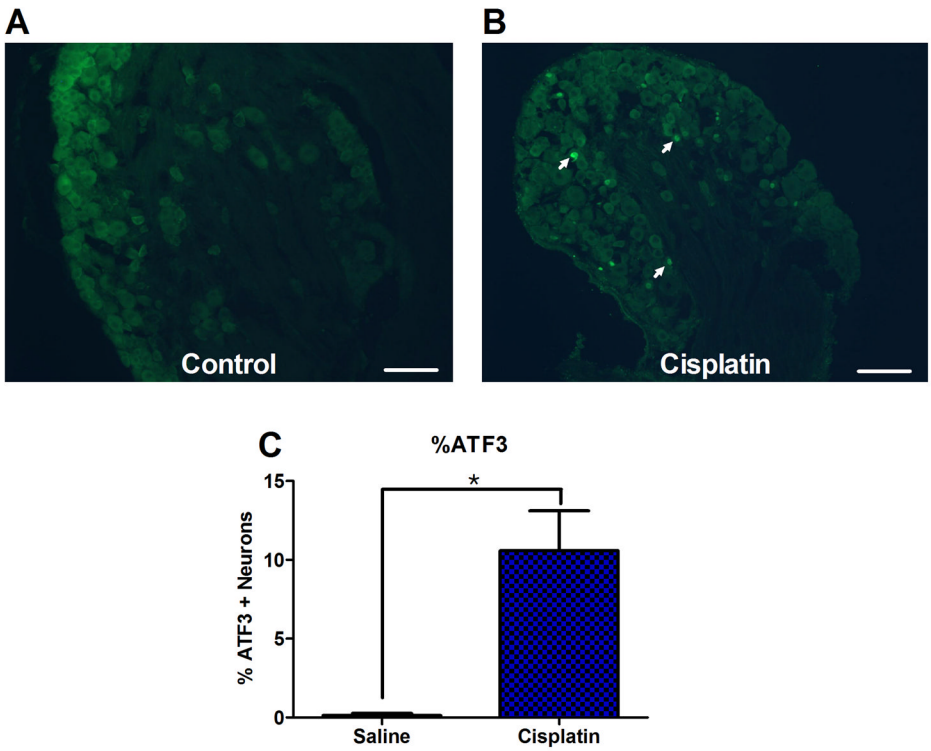

Fig. 6.

Figure presents immunohistochemistry for Activated Transcription Factor 3 (ATF3) (+) cell in the dorsal root ganglia (DRG) of control and cisplatin-treated mice at 30 days after initiation of treatment. A: In control animals ATF3(+) cells were few if not absent. B: In contrast, ATF3 staining was increased markedly in cisplatin-treated mice. C: In animals harvested 30 days after initiation of cisplatin treatment showed significant activation of neuronal ATF3 as compared to control group ($n = 4–5$ per group) (*$P < .05$). Scale bars represent 100 μm. Arrows indicate ATF3 (+) cells.