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Behavioral and pharmacological description of oxaliplatin-induced painful neuropathy in rat

Bing Ling [a,b], Nicolas Authier [a,b], David Balayssac [a], Alain Eschalier [b,c], François Coudore [a,b,c,*]

[a] *Université Clermont1, Faculté de Pharmacie, Laboratoire de Toxicologie, F63001 Clermont-Ferrand, France*
[b] *INSERM, U766, Faculté de Médecine et de Pharmacie, F63001 Clermont-Ferrand, France*
[c] *CHU Clermont-Ferrand, Service de Pharmacologie, Hopital G. Montpied, F-63003 Clermont-Ferrand, France*

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Abstract

We describe an animal model of nociceptive sensory neuropathy induced by repeat intravenous administration of oxaliplatin in which treated animals partly reproduce the characteristic pain symptoms in oxaliplatin-treated patients. We tested the ability of 1, 2 and 4 mg/kg oxaliplatin doses injected twice-weekly for four-and-a-half consecutive weeks to induce a nociceptive peripheral neuropathy in male Sprague–Dawley rats. The behavioral assessment revealed cold allodynia (10 °C) and hyperalgesia (4 °C) symptoms associated with a mechanical allodynia. The rats maintained a good general clinical status without motor dysfunction. The 2 mg/kg oxaliplatin dose and the tail-immersion test in cold water (10 °C) were selected to compare pharmacological sensitivity between single administered drugs as morphine, lidocaine, carbamazepine, gabapentin and repeated administration of drugs as clomipramine, venlafaxine, calcium and magnesium solutions. Magnesium solution (90 mg/kg) and venlafaxine (7.5 mg/kg) administration induced an antinociceptive effect whereas gabapentin (300 mg/kg), clomipramine (2.5 mg/kg) and lidocaine (3 and 6 mg/kg) only induced an antiallodynic effect.
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Keywords: Oxaliplatin; Cold allodynia; Cold hyperalgesia; Mechanical allodynia; Pharmacology

1. Introduction

Oxaliplatin is a third-generation platinum-based chemotherapy drug that has recently gained importance in the treatment of advanced metastatic colorectal cancer (Screnci et al., 2000; Baker, 2003). It has also been demonstrated that oxaliplatin exerts activities against various other cancers, including ovarian, breast and lung cancer (Muggia, 2004; Petit et al., 2006). Oxaliplatin is structurally similar to cisplatin but contains a 1,2-diaminocyclohexane carrier ligand. This modification enhances its anti-tumor activity but alters its side-effect profile. Since it is a platinum derivative, oxaliplatin induces neurotoxicity but no nephrotoxicity, as with cisplatin, and no hematotoxicity, as with carboplatin (Desoize and Madoulet, 2002).

Clinical practice describes a peripheral neurotoxicity characterized by paresthesia and dysesthesia in the distal extremities, spontaneous pain and loss of sensation which is considered a dose-limiting side-effect of oxaliplatin that can result from drug discontinuation. Oxaliplatin-induced neurotoxicity develops progressively in ≈10–15% of patients after a cumulative dose of 780–850 mg/m^2 (Gamelin et al., 2002; Wilson et al., 2002). There are few reports on the use of drugs to prevent or treat the painful symptoms

* Corresponding author. Tel.: +33 4 73 751 822; fax: +33 4 73 751 823.
E-mail address: fcoudore@chu-clermontferrand.fr (F. Coudore).

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of oxaliplatin-induced neuropathy in clinical practice. In general, two different strategies have been proposed: first, the ''stop-and-go'' strategy which uses the reversibility of symptoms to allow patients to stay on the oxaliplatin-containing first-line therapy for a more prolonged period (de Gramont, 2005), and second, the use of neuromodulatory agents. It is obvious that the stop-and-go strategy may affect the curative, but on the other hand, the use of neuromodulatory agents such as calcium-magnesium infusions, antiepileptics or antidepressants has low efficacy. This latter strategy has been based on clinical practice with only small sample sizes. Furthermore, most of these trials have only reported effects on acute neuropathy, and there is still no definitive proof of the efficacy of neuromodulatory interventions on painful oxaliplatin-induced cumulative toxicity.

Although the clinical symptoms produced by oxaliplatin are well described, no relevant animal model has yet been established for studying chronic oxaliplatin-induced neuropathy, as has previously been done for cisplatin-induced neurotoxicity (Authier et al., 2003). Cavaletti et al. (2001, 2002) only demonstrated chronic peripheral oxaliplatin-induced neurotoxicity as a decrease in sensory nerve conduction velocity induced by damage to neuronal cell bodies in the dorsal root ganglia, similarly to reports on other platinum-based drugs. Jamieson et al. (2005) created a model of neuropathy after repeated oxaliplatin administration for 8 weeks in order to investigate the histological modifications of nervous cells, but no behavioral pain tests were presented. Ghirardi et al. (2005) only used mechanical hyperalgesia symptoms in rats to describe oxaliplatin-induced neuropathy and test the efficacy of acetyl-L-carnitine.

The aim of this study was to behaviorally characterize nociceptive behaviors, as hyperalgesia and allodynia to thermal (cold and heat) and mechanical stimuli, after repeated oxaliplatin administration to rat. Afterwards the pharmacological efficiency of various drugs was assessed in order to outline the possibilities for treating these painful symptoms.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (Charles River, L'Arbresle, France) weighing between 150 and 175 g were housed (eight rats per treatment) in standard laboratory conditions with *ad libitum* access to food and water for at least one week before the experiments. The experiments were monitored by the local institution's Ethics Committee, and we followed IASP Committee for Research and Ethical Issues guidelines for animal research (Zimmermann, 1983). The researchers performing the behavioral studies were blinded with respect to the treatment administered.

2.2. Production of neuropathy

Oxaliplatin was generously provided by Debiopharm (Lausanne, Switzerland). It was dissolved in a 5% glucose solution at a concentration of 2 mg/ml depending on animal weight, to ensure i.v. injections into the lateral tail vein of less than 0.5 ml. Oxaliplatin was administered intravenously at one of three different doses, i.e. 1, 2 or 4 mg/kg, twice-weekly for four-and-a-half consecutive weeks. Volumes of a 5% glucose solution were adjusted to the weight of each rat and injected by the same route in the control group.

2.3. Assessment of general toxicity

Body weight was measured before each administration of oxaliplatin or vehicle and after the last administration, and up to day 21 following the last injection. All rats were examined daily for clinical signs such as piloerection or hind limb weakness, and to assess general health.

Motor activity was monitored every 7 days in a darkroom using an Actisystem actimeter (Apelex, Passy, France) over a 10-min interval before each injection and every 7 days until day 21 post-administration. A scoring system registered the activity of the animals through an electromagnetic system on a sensory plate. The number of counts was recorded automatically for 10 min.

Motor strength was tested using the Grip Strength test (Bioseb, Chaville, France), as follows. The rat was placed with both forepaws inside the front grip, and the strain gauge was zeroed. When the rat gripped the grid, it was steadily pulled backwards by the tail until its grip was broken. The reading on the strain gauge was recorded, the strain gauge was zeroed, and the rat was retested until three successive consistent results were obtained.

2.4. Behavioral studies

Behavioral tests representing different sensory components of neuropathic pain were conducted before each oxaliplatin injection and up to 21 days after the last oxaliplatin administration. Rats were habituated to handling by the investigator and to all the testing procedures during the week before the experiment. All tests were performed before oxaliplatin administration.

Mechanical allodynia was assessed using the von Frey Hair test (Tal and Bennett, 1994). Each rat was placed on an elevated plastic mesh in a clear plastic cage and allowed to adapt to the testing environment for at least 15 min. The von Frey Hairs (Semmes–Weinstein monofilaments, Stoelting IL, USA; 1.479, 2.041, 3.63, 5.495, 8.511, 11.749, 15.136 and 28.84 g) were applied to the plantar surface of each hind paw from below the mesh floor. For each filament, the test was repeated five times with an interval of 3–5 s between each stimulus. The threshold was determined as the lowest force that evoked a withdrawal response to one of the five stimuli.

Mechanical hyperalgesia was tested using the paw-pressure test (Randall and Selitto, 1957) which was carried out using a Ugo Basile analgesimeter (Apelex, Passy, France) measuring the pressure applied to the right hind paw before eliciting a vocalization from the animal. Cut-off pressure was 450 g.

Thermal hyperalgesia and allodynia were assessed using the tail-immersion test in water maintained at low (4 or 10 °C) or high (42 or 46 °C) temperature (Necker and Hellon, 1978). The duration of tail immersion was recorded, and a cut-off time of 15 s was used.

2.5. Pharmacological studies

The experiments were performed blind in a quiet room by a single experimenter using the method of equal blocks with randomization of treatments to avoid any uncontrollable environmental influence likely to induce a modification in behavioral responses. Animals presenting characteristic nociceptive symptoms and a good clinical status were used to test the antinociceptive effects of the drugs. According to previous results, consistent responses to cold non-noxious stimuli (10 °C) were observed after eight injections of oxaliplatin (2 mg/kg, i.v.). Hence, the tail-immersion test was used to test pharmacological effects just before and at various times after the antinociceptive drug treatment. Control rats were administered the relevant vehicle for each drug.

Morphine hydrochloride (Coopération Pharmaceutique Française, Melun, France) was dissolved in a solution of 0.9% sodium chloride on the day of the experiment and administered intravenously at 1, 2 and 4 mg/kg doses into the lateral tail vein (Kayser et al., 1995).

Lidocaine (Sigma, St Quentin Fallavier, France) was dissolved in a solution of 0.9% sodium chloride just before single intravenous administration at 1, 3 and 6 mg/kg doses.

Carbamazepine (Sigma, St Quentin Fallavier, France) was dispersed in a solution of 1% HPMC in 0.9% sodium chloride just before single intraperitoneal administration at 3, 10 or 30 mg/kg doses (Chapman et al., 1998).

Gabapentin (Neurontin®, Pfizer, Paris, France) was dissolved in sterile water just before single oral administration at 30, 100 and 300 mg/kg doses (Back et al., 2004).

Clomipramine (Sigma, St Quentin Fallavier, France) was dissolved in a solution of 0.9% sodium chloride just before five consecutive subcutaneous administrations of 2.5, 5.0 and 7.5 mg/kg doses at every half-life interval (2 h 35 min) (Ardid and Guilbaud, 1992).

Venlafaxine (Effexor®, Wyeth, Paris, France) was dissolved in a solution of 0.9% sodium chloride just before five consecutive subcutaneous administrations of 2.5, 5.0 and 7.5 mg/kg doses at every half-life interval (1 h) (Marchand et al., 2003).

Magnesium (magnesium chloride R.P. Normapur, Prolabo, Paris, France) was dissolved in sterile water and injected intraperitoneally in one, two or three doses of 30 mg/kg at 1-h intervals, giving total doses of 30, 60 and 90 mg/kg, respectively (Begon et al., 2002).

Calcium (calcium chloride R.P. Normapur, Prolabo, Paris, France) was dissolved in sterile water and injected intraperitoneally in one, two or three doses of 30 mg/kg at 1-h intervals, giving total doses of 30, 60 and 90 mg/kg, respectively.

2.6. Statistical analysis

Treatments were randomized within each cage. Behavioral data were examined using one-way analysis of variance (ANOVA) followed by Bonferroni *t*-test to detect differences between each treatment and control group at each time point. Pharmacological data were examined using one-way analysis of variance (ANOVA) followed when the *F* value was significant by Bonferroni *t*-test to compare the corresponding values of the drug-treated group with the vehicle group at each time point. (Statview 4.55, Abacas Concept Inc., Berkeley, CA, USA). Data were expressed as means ± standard error of the mean (SEM), and the levels of significance were set at: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

3. Results

3.1. Assessment of general toxicity

No deterioration in general status was observed, and clinical status remained good for the 1 and 2 mg/kg oxaliplatin-treated groups. No rats in the control group died during the course of the experiment. No alterations of body temperature and no abnormal clinical signs were observed. In the highest dose group (4 mg/kg), two rats died after injection 8 and two rats died after injection 9, while two other rats became thin after the end of the treatment.

No significant difference in weight gain compared to the control group was observed at any time in the 1 mg/kg group. A significant difference in weight gain compared with the control group was observed in the 2 and 4 mg/kg dose groups, with a maximal decrease of −11% and −18%, respectively, after injection 9 (Fig. 1A). Furthermore, there was no weight recovery after stoppage of the treatment.

No significant variation in motor activity was observed between 1, 2 and 4 mg/kg doses in the oxaliplatin-treated groups and the control group at any time during the course of the experiment.

No significant variation in motor strength compared to the control group was observed in the 1 mg/kg group at any time. In the 2 and 4 mg/kg dose groups, there was a significant decrease ($p < 0.05$ and $p < 0.01$, respectively) in motor strength after injection 8 in 75% and 100% of the rats, respectively, reaching −24% and −32%, respectively. All rats in the 2 mg/kg group completely recovered during the last 3 weeks of the experiment, whereas the surviving four rats of the 4 mg/kg group presented reduced motor strength up to the end of the test (Fig. 1B).

3.2. Behavioral studies

Before the first oxaliplatin injection, there were no significant differences in mean thresholds between the oxaliplatin-treated groups and control groups in any of the tests.

Mechanical allodynia (Fig. 2A): In the 1 mg/kg dose group, the withdrawal threshold decreased significantly compared with the control group after injection 4 in 100% of the rats ($p < 0.001$), with a maximum reduction

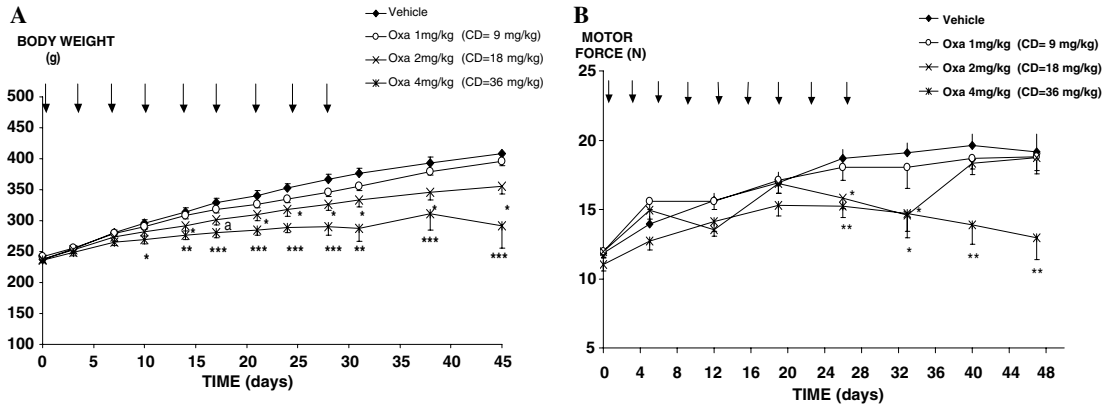

Fig. 1. (A and B) Evolution of body weight (A) and grip strength (B). The figure shows control (◆, vehicle, $n = 8$) and treated (o, oxaliplatin 1 mg/kg, $n = 8$); (×, oxaliplatin 2 mg/kg, $n = 8$); (ж, oxaliplatin 4 mg/kg, $n = 8$) rats. Each rat received two intravenous injections (↓) per week for 4.5 weeks. Scores were determined once a week for 7 weeks. Results are expressed as means (±SEM). A significant decrease ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, analysis of variance followed by Bonferroni t-test) in grip strength (B) was observed after injection 8 for the 2 and 4 mg/kg groups compared with the vehicle group, only the 2 mg/kg group completely recovered during the last 3 weeks of the experiment.

of −87%. The injection of 2 or 4 mg/kg doses significantly decreased the paw withdrawal thresholds after injection 4 ($p < 0.01$ and $p < 0.01$, respectively) with a maximum reduction of −89% and −81% ($p < 0.001$), which continued until the end of the experiment in the 1, 2 and 4 mg/kg dose groups.

Mechanical hyperalgesia (Fig. 2B): All rats injected with the 2 and 4 mg/kg doses showed a significant decrease in their vocalization thresholds compared with control group after injection 6 ($p < 0.01$ and $p < 0.01$, respectively), with a maximal reduction of −36% and −48%, respectively, after stoppage of the treatment. In contrast, there was no significant difference between the 1 mg/kg dose group and the controls.

Thermal allodynia

- A non-noxious cold stimulus (10 °C) (Fig. 3A) led to a reduced tail withdrawal latency in all dose groups. In the 1 and 2 mg/kg dose groups, there was a significant decrease after injection 6 ($p < 0.001$ and $p < 0.001$, respectively), reaching a maximum reduction of −89% after injection 8 ($p < 0.001$). For the 4 mg/kg doses, a similar significant decrease appeared in 88% of rats but only after injection 6 ($p < 0.01$), reaching a maximal reduction of −75% ($p < 0.001$). Tail withdrawal latency remained persistently low in all dose groups after injection 6 and up to the end of the experiment.

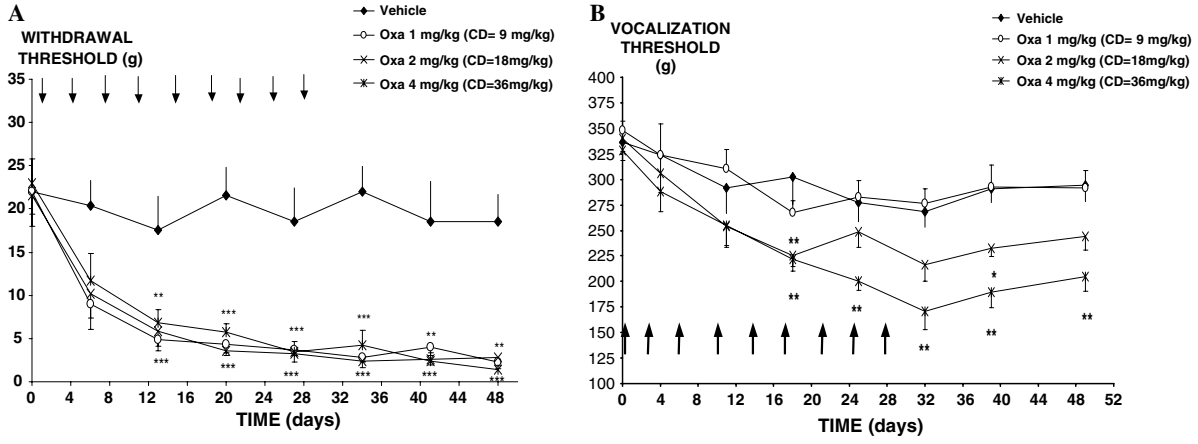

Fig. 2. (A and B) Mechanical allodynia (A) and hyperalgesia (B). Paw withdrawal threshold of control (◆, vehicle, $n = 8$) and treated (o, oxaliplatin 1 mg/kg, $n = 8$); (×, oxaliplatin 2 mg/kg, $n = 8$); (ж, oxaliplatin 4 mg/kg, $n = 8$) rats to von Frey hairs applied to the plantar surface of the hindpaw. Each rat received two intravenous injections (↓) per week for 4.5 weeks. Scores were determined once a week for 7 weeks. Results are expressed as means (±SEM). No significant variation was observed on control rats. A significant reduction ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$, analysis of variance followed by Bonferroni t-test) in mechanical allodynia (A) compared with the vehicle group was observed after injection 4 for the 1, 2 and 4 mg/kg groups and maintained at a maximum until the end of the experiment.

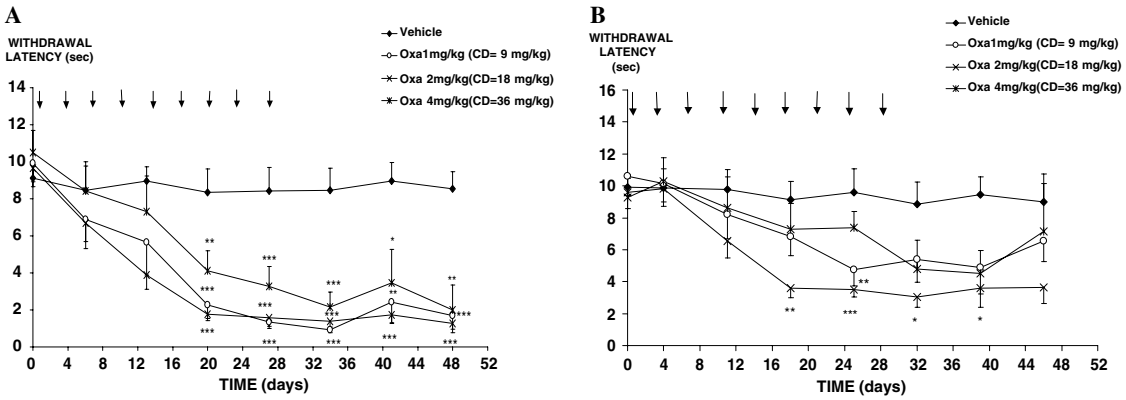

Fig. 3. (A and B) Cold (A) and heat (B) thermal allodynia. The figure shows tail withdrawal latencies of control (♦, vehicle, $n = 8$) and treated (o, oxaliplatin 1 mg/kg, $n = 8$); (×, oxaliplatin 2 mg/kg, $n = 8$); (ж, oxaliplatin 4 mg/kg, $n = 8$) rats after immersion of the tail in a cold (10 °C) or hot (42 °C) water bath. Each rat received two intravenous injections (↓) per week for 4.5 weeks. Scores were determined once a week for 7 weeks. Results are expressed as means (±SEM). No significant variation was observed on control rats. A significant difference (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, analysis of variance followed by Bonferroni t-test) in cold allodynia (A) was observed after injection 4 for the 1 and 2 mg/kg groups compared with the vehicle group and after injection 6 for the 4 mg/kg group. All dose groups remained persistently low values after injection 6 throughout the experiment.

- A non-noxious hot stimulus (42 °C) (Fig. 3B) led to a significantly reduced tail withdrawal latency after injection 6 ($p < 0.01$) in 100% of the rats in the 2 mg/kg dose groups, reaching a maximum reduction of −66% and remaining stable until the end of the experiment. In the 1 mg/kg group, a significantly lower tail withdrawal latency was observed only after injection 8 in 86% of the rats ($p < 0.01$), and began to recover the last week of the experiment.

Thermal hyperalgesia

- A cold noxious stimulus (4 °C) (Fig. 4A) led to a significantly reduced tail withdrawal latency compared to controls in 100% of the rats in the 1 and 2 mg/kg dose groups after four oxaliplatin injections ($p < 0.001$ and $p < 0.001$, respectively). In the 4 mg/kg dose groups, there was a significant decrease ($p < 0.05$) after injection 4 in 100% of the rats, reaching a maximum reduction of −89% ($p < 0.001$). All latency values fell and then plateaued from injections 4 through to the end of the study.
- A hot noxious stimulus (46 °C) (Fig. 4B) led to a significantly reduced tail withdrawal latency only in the 2 mg/kg dose group in 88% of rats ($p < 0.01$) after injection 8, reaching a maximum reduction of −47% ($p < 0.01$), with incomplete recovery over the last week of the experiment.

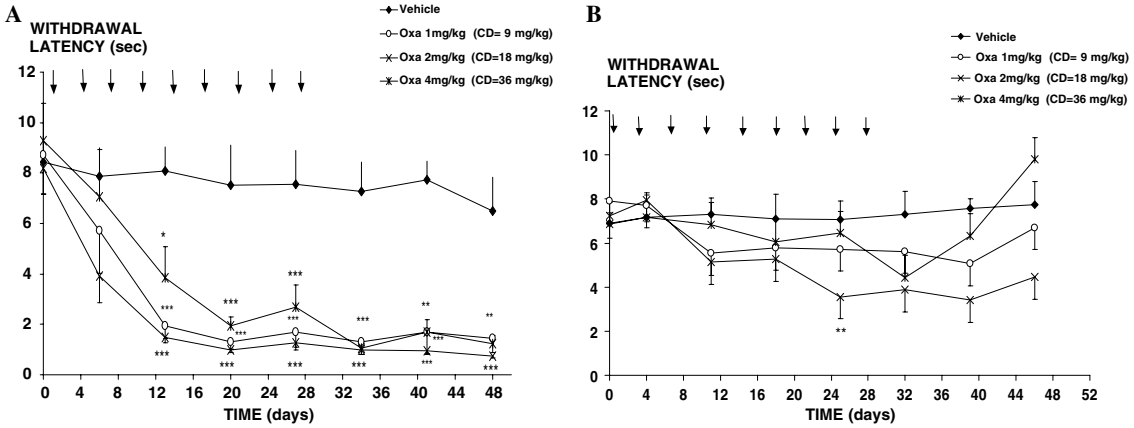

Fig. 4. (A and B) Cold (A) and heat (B) thermal hyperalgesia. The figure shows tail withdrawal latencies of control (♦, vehicle, $n = 8$) and treated (o, oxaliplatin 1 mg/kg, $n = 8$); (×, oxaliplatin 2 mg/kg, $n = 8$); (ж, oxaliplatin 4 mg/kg, $n = 8$) rats after immersion of the tail in a cold (4 °C) or hot (46 °C) water bath. Each rat received two intravenous injections (↓) per week for 4.5 weeks. Scores were determined once a week for 7 weeks. Results are expressed as means (±SEM). No significant variation was observed on control rats. A significant difference (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, analysis of variance followed by Bonferroni t-test) in cold hyperalgesia was observed after injection 2 for the 2 mg/kg group and after injection 4 for the 1 and 4 mg/kg groups compared with the vehicle group. All dose groups remained persistently low values after injection 4 or 5 throughout the experiment.

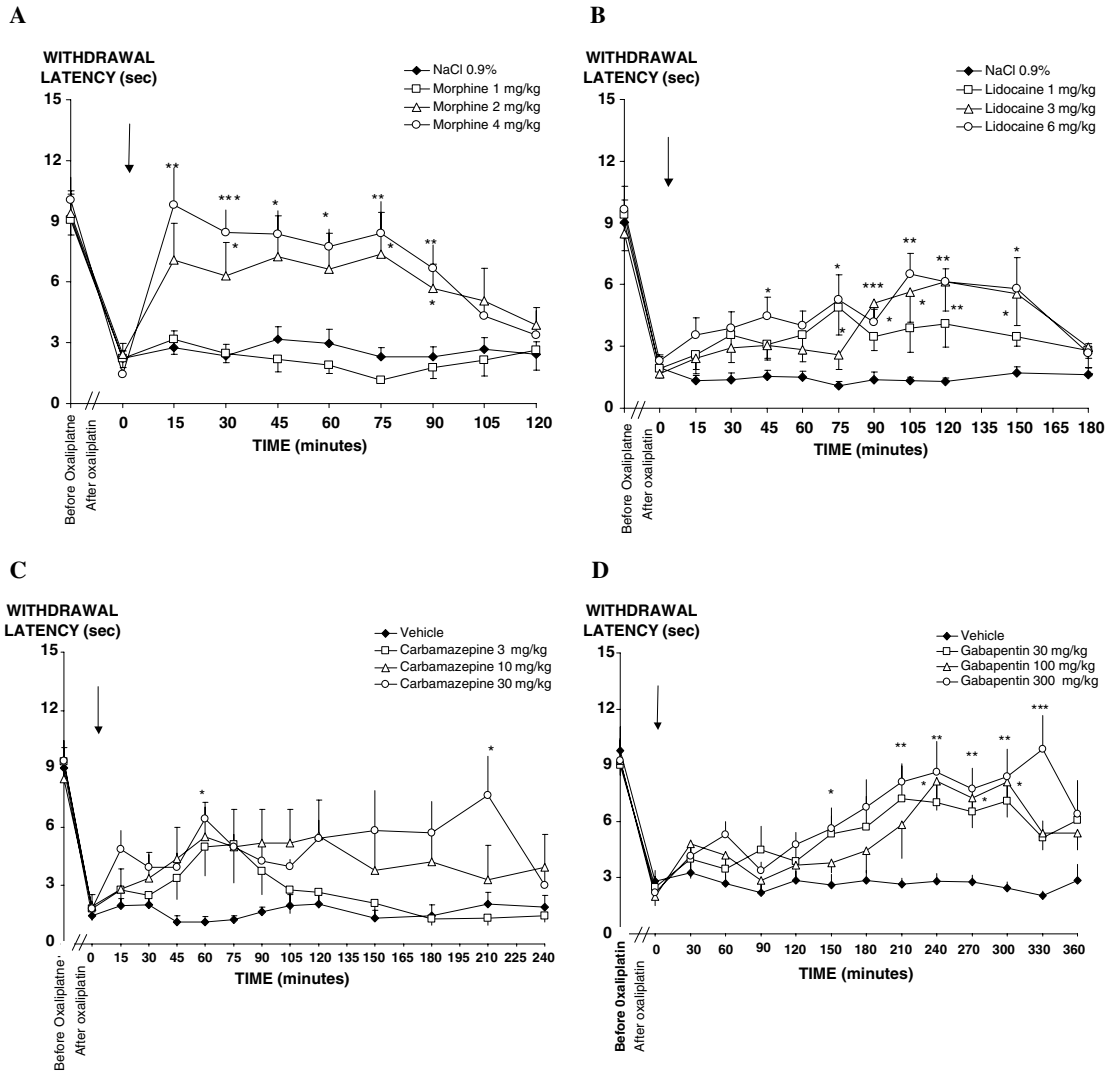

Fig. 5. (A–D) Pharmacological effects of single administration of morphine (A), lidocaine (B), carbamazepine (C), gabapentin (D) or saline (◆, NaCl 0.9%): (A) i.v. morphine (□, 1 mg/kg; △, 2 mg/kg; ○, 4 mg/kg); (B) i.v. lidocaine (□, 1 mg/kg; △, 3 mg/kg; ○, 6 mg/kg); (C) i.p. carbamazepine (□, 3 mg/kg; △, 10 mg/kg; ○, 30 mg/kg); (D) p.o. gabapentin (□, 30 mg/kg; △, 100 mg/kg; ○, 300 mg/kg) on tail withdrawal latencies using the tail immersion test in cold non-noxious (10 °C) water in a rat model of oxaliplatin-induced neuropathy (2 mg/kg, i.v. two times per week). The arrow corresponds to the injection or saline. Values were determined before and after oxaliplatin-induced neuropathy and every 15–30 min for 120 min (A), 180 min (B), 240 min (C) and 360 min (D) after drug injection. Results are expressed in seconds as means ± SEM. $n = 8$ in each group. There were no significant variations in control rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, *versus* the corresponding pre-drug values (analysis of variance followed by Bonferroni *t*-test).

Whatever the temperature applied, the control group did not show any significant variation in tail withdrawal latencies at any point in the study.

3.3. Pharmacological results

The basal value for the decrease in tail withdrawal latency was around 2.0 ± 0.2 s.

- Morphine – single intravenous administration (Fig. 5A): Only the 2 and 4 mg/kg doses induced a significant increase in tail withdrawal latency (in 50% and 88% of rats, respectively) between 15 min (+250%) and 75 min (+261%) post-administration compared with the saline group. The strongest effect occurred 15 min after the 4 mg/kg morphine injection and persisted for 60 min.
- Lidocaine – single intravenous administration (Fig. 5B): The 3 and 6 mg/kg doses induced a dose-dependent increase in tail withdrawal latency compared to the saline group. The maximum effect appeared at 120 min (+377%) and 105 min (+389%) in 75% and 86% of rats, respectively.
- Carbamazepine – single intraperitoneal administration (Fig. 5C): No significative effect was observed with all three doses.

- Gabapentin – single oral administration (Fig. 5D): Tail withdrawal latency significantly increased at the 300 and 100 mg/kg dose, after 150 min ($p < 0.05$) and after 240 min ($p < 0.05$), respectively, with a maximum effect at 330 min post-administration (+388%) in 87% of rats injected with 300 mg/kg dose.
- Clomipramine – repeat subcutaneous administration (Fig. 6A): The lower dose of clomipramine, i.e. 2.5 mg/kg, induced a significant ($p < 0.01$) increase in tail withdrawal latency in 100% of rats between 90 and 180 min post-administration, with a peak effect at 90 min (+342%).
- Venlafaxine – repeat subcutaneous administration (Fig. 6B): The 7.5 mg/kg dose induced a significant ($p < 0.01$) antinociceptive effect at 45 min post-administration, and the effect continued to increase until 90 min post-administration (389%, respectively). The effect of venlafaxine was dose-dependent and lasted for at least 135 min.
- Magnesium – repeat intraperitoneal administration (Fig. 6C): A triple injection (30 mg/kg each) induced a significant antinociceptive effect from 15 min post-administration ($p < 0.05$) and increasing up to 75 min (+383%, $p < 0.001$). The pharmacological effect lasted for at least 120 min. A similar tendency was observed after both single and double injections (30 mg/kg each).
- Calcium – repeat intraperitoneal administration (Fig. 6D): The triple calcium injection (30 mg/kg each) induced a significant maximal antinociceptive

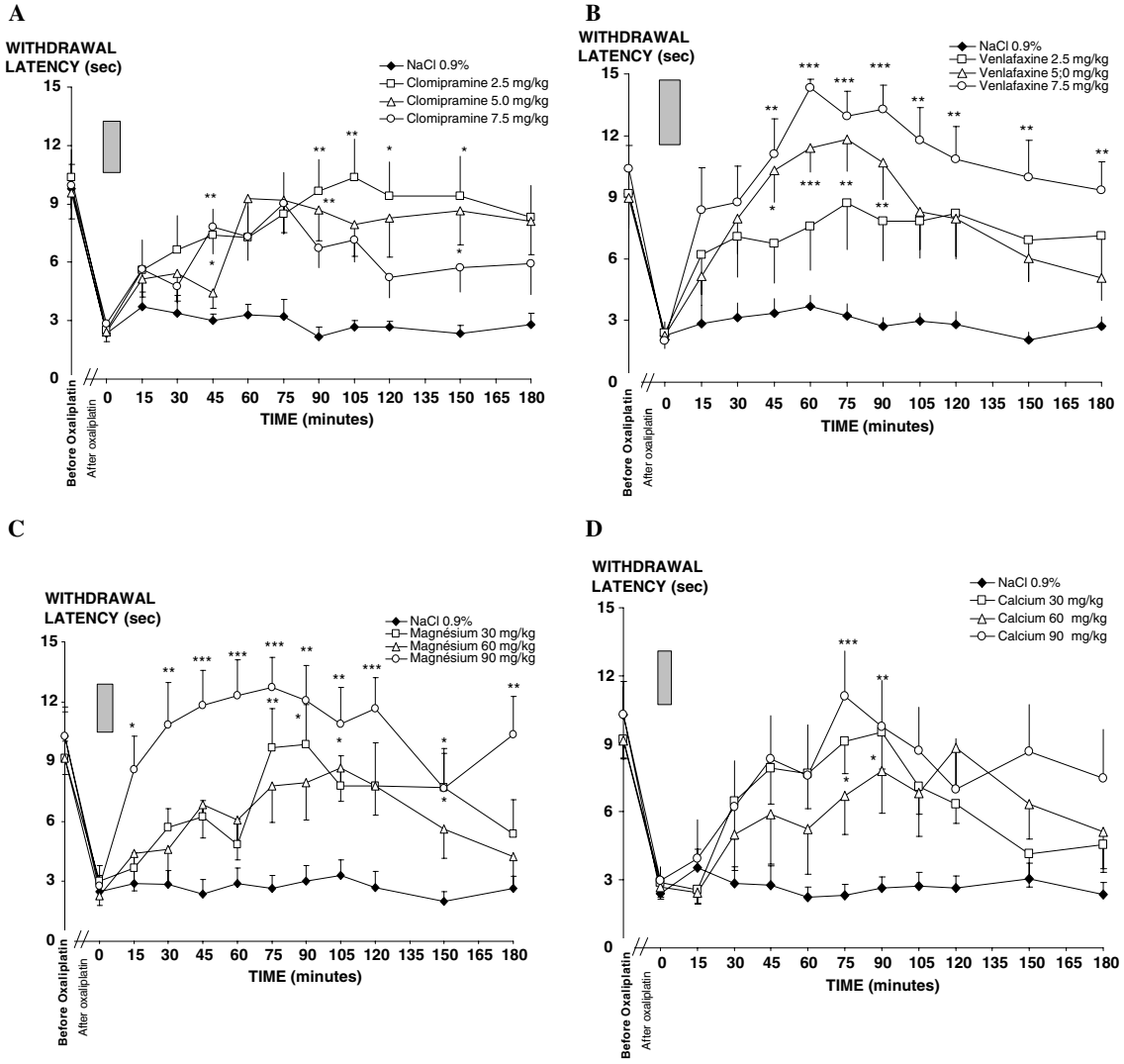

Fig. 6. (A–D) Pharmacological effects of repeated administration of clomipramine (A), venlafaxine (B), magnesium (C), calcium (D) or saline (◆, NaCl 0.9%). (A) s.c. clomipramine (□, 2.5 mg/kg; △, 5 mg/kg; ○, 7.5 mg/kg); (B) s.c. venlafaxine (□, 2.5 mg/kg; △, 5 mg/kg; ○, 7.5 mg/kg) ; (C) i.p magnesium (□, 30 mg/kg; △, 60 mg/kg; ○, 90 mg/kg); (D) i.p. calcium (□, 30 mg/kg; △, 60 mg/kg; ○, 90 mg/kg) on tail withdrawal latencies using the tail immersion test in cold non-noxious (10 °C) water in a rat model of oxaliplatin-induced neuropathy (2 mg/kg, i.v. two times per week). The arrow corresponds to the injection or saline. Values were determined before and after oxaliplatin-induced neuropathy and every 15 min for 180 min after drug injection. Results are expressed in seconds as means ± SEM. $n = 8$ in each group. There were no significant variations in control rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, *versus* the corresponding pre-drug values (analysis of variance followed by Bonferroni *t*-test).

effect 75 min after the last injection (+381%, $p < 0.001$).

4. Discussion

In this study, rats chronically administered oxaliplatin showed significant behavioral nociceptive signs which are consistent with clinical symptoms especially cold hypersensitivity taking in account that some clinical signs, as spontaneous pain, cannot be accurately assessed in animals. No relevant animal model of nociception has yet been reported for oxaliplatin. Studies published to date have reported histological or electrophysiological alterations or mechanical hyperalgesia in rats (Holmes et al., 1998; Cavaletti et al., 2001, 2002; Ghirardi et al., 2005; Jamieson et al., 2005).

The absence of deterioration and variation in motor activity after oxaliplatin injections allowed us to validate the behavioral tests. Half of the 4 mg/kg dose group died after the 8–9th intravenous injection, when cumulated dose reached 32 mg/kg. This dose was higher than that used by Cavaletti et al. (2002) who injected a 2–3 mg/kg dose of oxaliplatin twice weekly until nine injections.

Our study reports major behavioral effects of oxaliplatin administration on nociceptive thresholds induced by both noxious and non-noxious stimuli. In response to cold stimuli (10 and 4 °C), rapid decreases in nociceptive thresholds were observed in all dose groups that remained persistently low, reaching a maximum reduction of −89% after the 4 or 6th injection. In response to hot stimuli (42 and 46 °C), a lower maximum reduction in nociceptive threshold of −58% was only observed in the 2 mg/kg group after the 6–8th injection. In response to non-noxious mechanical stimuli, all dose groups showed significant decreases in pain thresholds after the 4th injection.

The oxaliplatin-induced neuropathy model is less well characterized than the cisplatin-induced animal model of neuropathy (Cavaletti et al., 1992; Authier et al., 2003). Symptoms of cisplatin neurotoxicity are most likely to be chronic, whereas symptoms of oxaliplatin neurotoxicity are both acute and chronic. As with all platinum-based drugs, the resulting peripheral neuropathies are characterized by paresthesia and dysesthesia in the distal extremities, spontaneous pain and loss of sensation (Mathe et al., 1986; Extra et al., 1990; Ibrahim et al., 2004). However, oxaliplatin-induced cold allodynia and hyperalgesia symptoms are nevertheless different, since they are more rapidly and completely reversible than cisplatin-induced symptoms. Furthermore, our animal model showed different intensities of cold allodynia and hyperalgesia signs to those observed in cisplatin-induced neuropathy. Compared to previous findings (Authier et al., 2003), oxaliplatin-induced cold hyperalgesia and allodynia signs were more than twofold stronger than those of cisplatin (−87% vs. −42% and −85% vs. −30%, respectively), whereas all other nociceptive symptoms presented similar intensity. Holmes et al. (1998) compared cisplatin- (2 mg/kg i.p.) and oxaliplatin-induced (4 mg/kg i.p., twice weekly for 4.5 weeks) histological modifications. Cisplatin-induced morphometric changes were higher than or equal to oxaliplatin changes. Cavaletti et al. (2001, 2002) and Holmes et al. (1998) showed ganglia nucleolar, nuclear and somatic size reduction in the dorsal root with nucleolar segregation induced by damage to cell bodies in dorsal root ganglia. Jamieson et al. (2005) reported that oxaliplatin causes selective atrophy of subpopulations of dorsal root ganglion neurons without cell loss. Oxaliplatin slowed nerve conduction velocity and delayed conduction times in peripheral sensory nerves without affecting central or motor nerve conduction. The decrease in sensory nerve conduction velocity and damage to neuronal cell bodies in the dorsal root ganglia were nevertheless similar to those reported with other platinum-based drugs. The different pathophysiologies of the neurotoxic alterations suggest different targets for cisplatin- and oxaliplatin-induced neuropathy.

It has been suggested that oxaliplatin may block voltage-gated sodium channels (Adelsberger et al., 2000). Morphologic changes in the dorsal ganglia and sciatic nerve have been reported in other in vivo chronically induced neuropathy models (Holmes et al., 1998; Luo et al., 1999; Cavaletti et al., 2001). The predominant action is expressed in A-fibers, since interferences with sodium channels are expressed more strongly in A-fibers than in C-fibers. Moreover, Adelsberger et al. (2000) showed that the subset of dorsal root ganglia cell bodies which were insensitive to oxaliplatin were cell bodies of C-fiber axons. This specific action on A-fibers could explain the abnormal reactions to cold stimuli.

Clinical practice is able to propose a wide range of strategies to prevent or treat oxaliplatin-induced neurotoxicity via conventional pain therapies such as morphine, lidocaine, carbamazepine, gabapentin, clomipramine or venlafaxine (Screnci et al., 2000; Carrato et al., 2002; Gamelin et al., 2002, 2004) as well as by calcium or magnesium (Cersosimo, 2005; Durand et al., 2005; Grothey, 2005). As in our studies the behavioral responses were maximal for cold stimuli and the main clinical symptom was hypersensitivity to non-noxious stimuli, only animals which showed allodynia symptoms to cold were used to test the antinociceptive effects of drugs.

Literature data indicate that either a single administration (morphine, lidocaine, carbamazepine, gabapentin) or repeat injections performed at every half-life interval ($t1/2 = 2$ h 35 and 1 h in rats for clomipramine and venlafaxine, respectively) are efficient in rats (Ardid and Guilbaud, 1992; Marchand et al., 2003). As

described for magnesium chloride in rats (Begon et al., 2002), the repetition of injections may make it possible to reach sufficiently high concentrations in the central nervous system to obtain an analgesic effect.

We classified all the tested drugs by the order of their analgesic activity using two parameters: the ratio of the difference (AUC of drug effect–AUC of excipient effect) to the AUC of vehicle effect, and the ratio of the difference (maximum drug effect–maximum excipient effect) to the maximum excipient effect.

The strongest antinociceptive activities were observed following repeat administration of magnesium or venlafaxine. Based on our data, magnesium administration may be a good option for treating chronic pain. Magnesium administration has been clinically demonstrated as an effective and convenient means of treating and reducing the severity of neuropathic symptoms (Gamelin et al., 2004). The incidence and intensity of grade 3 distal paresthesia were described as lower in the Ca/Mg group than in the control group, with 20% of patients in the Ca/Mg group versus 45% in the control group presenting neuropathy at the end of the treatment, and Ca/Mg-treated patients recovered significantly more rapidly from neuropathy.

Venlafaxine administration induced a significant antinociceptive effect on cold allodynia. This treatment modality mimics the plasma half-life of venlafaxine in rats and has previously been shown to induce a significant antihyperalgesic effect in Chronic Constriction Injury (CCI), streptozocin- and vincristine-induced neuropathy models (Marchand et al., 2003). The use of venlafaxine to treat oxaliplatin-induced pain has been described previously (Durand et al., 2005).

A single oral administration of gabapentin induced an anti-allodynic effect. The literature data describe the effect of gabapentin (30–100 mg/kg i.p.) as an attenuation of cold allodynia in other models of nerve injury, even if different modes of thermal stimulus can elicit different responses (Chapman et al., 1998; Fox et al., 2003; Back et al., 2004; Blackburn-Munro and Erichsen, 2005). The efficacy of gabapentin in preventing and treating oxaliplatin neurotoxicity has been clinically confirmed (Mariani et al., 2000).

Among the single administration drugs, the 2 and 4 mg/kg i.v. doses of morphine and the 3 and 6 mg/kg i.v. dose of lidocaine all showed an effective anti-allodynic effect in oxaliplatin-induced neuropathy. However, none of these drugs had an antinociceptive effect since they only restored the basal values. The anti-allodynic efficacy of morphine has not been clearly established. In the CCI model, an i.v. morphine dose of 1 mg/kg resulted in an antinociceptive effect in mechanical tests (Kayser et al., 1995). The reported anti-allodynic effect of lidocaine reflected high sensitivity of sodium channels (Akopian et al., 1999). Absence of response to cold allodynia at 10 °C under systemic lidocaine (0.6–1.8 mg/kg i.v.) has previously been reported in a CCI model (Jasmin et al., 1998).

Clomipramine also induced an anti-allodynic effect, but to a lesser extent, and the maximum effect was obtained at the lowest dose (2.5 mg/kg). In contrast, some other antidepressant serotonin and noradrenaline reuptake inhibitors such as imipramine (10 mg/kg i.p.) failed to significantly attenuate cold allodynia in rats following avulsion of the brachial plexus (Rodrigues-Filho et al., 2004), and amitriptyline at the same dose was shown to be uneffective against mechanical allodynia in a rat model of spinal nerve ligation (Esser and Sawynok, 1999).

In this study, we characterized the neurotoxic profile of oxaliplatin in rats, especially cold hypersensitivity with allodynia and hyperalgesia, and mechanical allodynia. Based on our data, magnesium chloride and venlafaxine, via monoaminergic system (Lang et al., 1996) (Marchand et al., 2003), presented significant antinociceptive activities and may be a good choice for treating chronic oxaliplatin-induced neuropathy in humans. The hypothesis of channelopathy for oxaliplatin-induced allodynia is confirmed by the good action of the magnesium solution, partly mediated through NMDA receptors (Wilson et al., 2005). Our experimental results are in accordance with those of Gamelin et al. (2004) and Durand et al. (2005), and emphasize once more the possibility of treating oxaliplatin-induced cold allodynia symptoms with either magnesium solution or venlafaxine.

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