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Behavioral and immunohistological assessment of painful neuropathy induced by a single oxaliplatin injection in the rat

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Abstract

In clinical use, a single infusion of oxaliplatin, widely used to treat metastatic colorectal cancer, induces specific sensory neurotoxicity signs triggered or aggravated by exposure to cold. To study the pathophysiology of these symptoms, we developed and characterized an animal model that reproduces the effects of a single intraperitoneal oxaliplatin administration (3, 6 and 12 mg/kg). Significant allodynia and hyperalgesia to cold stimuli were rapidly observed from 24 h to day 5 with a maximum lowering of 76% at $t + 30$ h versus control. Other behavioral assessments revealed rapid persistent mechanical allodynia, but no thermal hyperalgesia or allodynia to heat and no hyperalgesia to mechanical stimuli. An immunohistochemical study in the superficial layers of the spinal dorsal horn revealed a marked increase in substance P immunoreactivity versus controls (12% versus 4%), whereas calcitonin gene-related peptide (CGRP) immunoreactivity was unchanged. This new animal model for the first time closely mimics the effects observed in humans after a single oxaliplatin infusion, especially onset and highly intense sensory disturbances, hypersensitivity to cold with allodynia and hyperalgesia signs. This model may help to elucidate the mechanisms of this thermal hypersensitivity, especially the possible involvement of small-diameter A-fibers in cold allodynia symptoms. These selective effects may clue up the mechanistic basis for the acute oxaliplatin neuropathy leading to a better understanding of the clinical condition and to optimize its treatment.

Keywords: Anticancer drug; Hyperalgesia; Allodynia; Substance P; CGRP

1. Introduction

Oxaliplatin is a third-generation platinum-based chemotherapy drug that has gained importance in the treatment of advanced metastatic colorectal cancer (Screnci et al., 2000; Baker, 2003). Oxaliplatin is active 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. Being a platinum derivative, oxaliplatin induces neurotoxic effects, but no nephrotoxicity

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as with cisplatin and no hematotoxicity as with carboplatin are observed (Desoize and Madoulet, 2002).

About 85 to 95% of oxaliplatin-treated patients rapidly develop significant pain signs without motor dysfunction during the oxaliplatin infusion period, peaking in the first 24–48 h (Extra et al., 1990; Mathe et al., 1986; Ibrahim et al., 2004; Cersosimo, 2005). This acute neurotoxicity is characterized by the rapid onset of cold-induced distal dysesthesia, paresthesia, hypoesthesia, dysesthesia of the hands, feet, peri-oral area or throat (Gamelin et al., 2002; Lehky et al., 2004). The symptoms disappear in about a week. Although all the other platinum-based chemotherapeutic agents cause sensory neuropathy after chronic treatment, none produces such acute painful signs. Only little or no neuronal degeneration was observed in patients suffering from these sensory symptoms, suggesting a specific effect of oxaliplatin on sensory neurons and (or) motor neurons or muscle cells (Grothey, 2003).

Although the clinical painful signs produced by chronic oxaliplatin treatment have been described in animals (Ling et al., 2007), the acute oxaliplatin-induced signs have never been displayed and studied in animal models. Using an animal model of chronic oxaliplatin-induced neuropathy, Cavaletti et al. (2001, 2002) showed a decrease in sensory nerve conduction velocity induced by damage to neuronal cell bodies in the dorsal root ganglia (DRG), similar to those seen with other platinum-based drugs. We previously reproduced in rats the nociceptive signs observed in humans after repeated oxaliplatin injections (Ling et al., 2007). The following behaviors observed in animals are considered to indicate nociception: hyperalgesia, i.e. strong withdrawal responses to a moderate heat stimulus and allodynia, i.e. withdrawal in response to non-noxious tactile or cold stimuli (8–28 °C). Other authors have investigated the histological modifications of nerve cells (Jamieson et al., 2005) or have shown mechanical hyperalgesia in rats (Ghirardi et al., 2005).

The aim of this study was to develop and validate a new neuropathic rat model of a single administration of oxaliplatin reproducing the characteristic pain signs observed in oxaliplatin-treated patients with metastatic colorectal cancer. Given that the development of behavioral symptoms, such as hyperalgesia and allodynia is classically linked to release of substance P (SP) and calcitonin gene-related peptide (CGRP) by the primary sensory afferents of the spinal dorsal horn, it made sense to look for possible changes in neuropeptide release after a single injection (Yoon et al., 2003). This specific animal model may thus help to give insight into the mechanisms involved and assess the pharmacological efficacy of drugs that could be used to treat this severe painful neuropathy, as carbamazepine or magnesium (Gamelin et al., 2002).

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 (four rats per cage and eight rats per treatment) in standard laboratory conditions (temperature-controlled environment with a light–dark cycle of 12:12 h) with *ad libitum* access to food and water for at least 1 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. Chemicals

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 intraperitoneal injections of less than 2.5 ml. Oxaliplatin was intraperitoneally administered alone at one of three different doses, namely 3, 6 or 12 mg/kg (Holmes et al., 1998; Cavaletti et al., 2001). 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 and up to 10 days after the administration of oxaliplatin or vehicle. All the rats were examined daily for symptoms, such as motor dysfunction, piloerection or temperature, and to assess general health. Body temperature was measured at the same time with an infrared ear thermometer.

2.4. Behavioral examination

Behavioral tests representing different sensory components of neuropathic pain were conducted before oxaliplatin and after 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 to assess baselines.

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. Successively greater diameter von Frey nylon monofilaments (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 medial

plantar surface of hind paw from below the mesh floor. For each filament, a series of five stimuli were applied with an interval of 3–5 s per paw. 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). Nociceptive thresholds, expressed in grams, were measured by applying increasing pressure to the right hind paw using an Ugo Basile analgesimeter (Apelex, Passy, France). The parameter used to quantify the nociceptive threshold was the vocalization of the animal. Rats were habituated to the testing procedures and handling by the investigator during the week prior to the experiment. Experiments were performed until two similar consecutive pressure values were obtained. 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; Allchorne et al., 2005). The tail of rat was immersed in water maintained until the tail was withdrawn. The duration of tail immersion was recorded, and a cut-off time of 15 s was used. Rats were habituated to the testing procedures and to handling by the investigator during the week prior to the experiment.

To accurately approach hypersensitivity to cold and reproduce the early symptoms of administration in patients, a 10 °C tail-immersion test was conducted from 0 to 120 h (until a partial recovery was observed in the treated groups) after a single oxaliplatin injection. Two series of rats were measured at $t+0$, 2, 6, 12, 24, 30, 36, 48, 60, 72, 84, 96, 108, 120 h and $t+0$, 12, 14, 16, 20, 24, 30, 36, 48, 60, 72, 84, 96, 108, 120 h, respectively. In addition, other nociceptive behavioral tests were performed every 24 h for 10 days (partial recovery in the treated groups).

2.5. Immunohistochemistry

All procedures were approved by the local ethical committee and complied with the policy of the Society of Neuroscience on the use of animals in neuroscience research. All chemicals and reagents were used according to the DakoCytomation instructions.

Within 24 h after the oxaliplatin injection, the rats used for immunohistochemical studies were separated from those used for behavioral testing. All chemicals were of analytical grade. Animals were deeply anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.), perfused intracardially by freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS) (pH 7.4). After perfusion, L4-5 lumbar spinal cord was removed, post-fixed in the same fixative for 1 h at 4 °C and then transferred to 50% ethanol. The L4-5 spinal cord was identified by identification of the lumbar enlargement and nerve roots.

Sections of the lumbar spinal cord were paraffin-embedded and the next day serially sectioned at 5 μm thickness in a transverse plane with a microtome. Tissue sections were then mounted on slides. Sections were deparaffined (xylene 3 min × 5 min, ethanol 100% 2 min × 10 min, ethanol 95% 2 min × 10 min and distilled water 2 min × 5 min). Deparaffinized sections were microwaved in 10 mM citrate buffer (pH 6.1) to unmask antigenic epitopes for 10 min at 98 °C and allowed to cool for 30 min at room temperature. Sections were washed for 5 min in distilled water and then in TBS Tween NaF. Next the sections were then hooped with a Dako pen. Sections were washed for 10 min in peroxidase-blocking solution (DakoCytomation®) and washed for 10 min in TBS Tween NaF. The sections were then incubated in primary polyclonal goat CGRP antibody (SC-8856, 1: 200, Santa Cruz Biotechnology®, Tebu-bio, France) and primary polyclonal goat Substance P antibody (SC-9758, 1:500, Santa Cruz Biotechnology®, Tebu-bio, France) at 4 °C overnight. Sections were then washed in TBS Tween for 15 min and incubated for 30 min at room temperature in biotinylated swine anti rabbit/mouse/goat antibody (Universal LSAB® + Kits DakoCytomation®). After another wash in PBS, tissue sections were incubated with a streptavidin (Universal LSAB® + Kits DakoCytomation®) for 30 min. After a final wash in TBS Tween, tissue sections were covered with the chromogen diaminobenzidine (Universal LSAB® + Kits DakoCytomation®) for 5 min and then rinsed in distilled water for 2 min × 5 min.

We placed the sections in aqueous mounting medium (Kits DakoCytomation®) and examined them with a Nikon Labophot-2 microscope (Nikon®, Tokyo, Japan). After focusing we photographed the dorsal horn. Resolution, brightness and contrast of the images were optimized using Adobe Photoshop 7.0 (Adobe Systems Inc.®, San Jose, CA).

The density of fibers immunoreactive for substance P or CGRP in the superficial dorsal horn laminae was determined with an image analysis system. Sections were viewed on a Nikon Labophot-2 microscope with a 100× magnification for CGRP- and substance P immunostaining. We then used LUCIA® software (Cytogen GmbH, Sinn-Fleisbach, Germany). Immunoreactive structures (fibers and varicosities) were separated from the background by an interactive method of thresholding and converted into black and white digital images. All the objects in a fixed area (250 μm × 150 μm) were automatically counted. The surface occupied by immunostained fibers was expressed as the ratio of the number of black pixels to the total number of pixels in the selected area.

2.6. Statistical analysis

Treatments were randomized within each cage. Behavioral data were examined using repeated-measures analysis of variance (ANOVA) followed, when the F-value was significant, by a Bonferroni t-test to compare the corresponding values of each treatment group with the vehicle group at each time point. (Statview 4.55, Abacas Concept Inc., Berkeley, CA, USA). Data were expressed as mean ± standard error of the mean (S.E.M.), and the levels of significance were set at $*p<0.05$, $**p<0.01$ and $***p<0.001$.

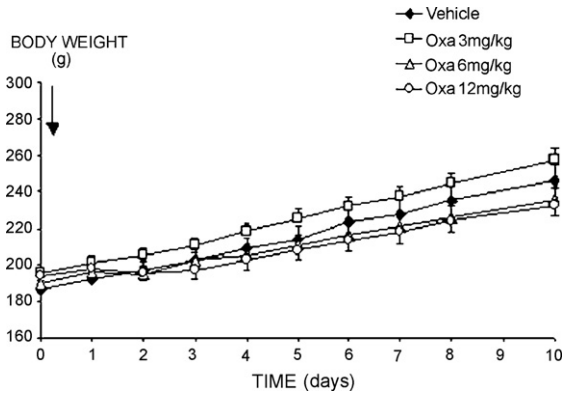

Fig. 1. Evolution in body weight. The figure shows control (♦, vehicle, $n=8$) and treated (□, oxaliplatin 3.0 mg/kg, $n=8$); (△, oxaliplatin 6.0 mg/kg, $n=8$); (◯, oxaliplatin 12 mg/kg, $n=8$) rats. Each rat received a single intraperitoneal injection (↓). Results are expressed as mean ± S.E.M. Statistical analyses were performed using analysis of variance followed by a Bonferroni *t*-test (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

The density of fibers immunoreactive for substance P or CGRP in the superficial dorsal horn laminae was expressed as the ratio of the number of black pixels to the total number of pixels in the selected area. Data from immunohistological experiment were examined using the Student *t*-test to detect differences between the treated and the vehicle group.

3. Results

3.1. Assessment of general toxicity

No rat died in the oxaliplatin groups irrespective of the dose used during the course of the experiment. Fig. 1 demonstrates the evolution of weight gain during the experiment. No significant difference in weight gain was observed after single injection throughout the course of the experiment compared with the control group. From day 1 to 3, the rats receiving 12 mg/kg showed no body weight gain compared with the control group, and at the end of the study an insignificant difference versus control rats was observed (−6%). Temperature variations were checked 2 h after the injection of oxaliplatin, and were not significant. For the 3 and 6 mg/kg oxaliplatin-treated groups, no deterioration in general status was observed, and clinical status remained good. However rats displayed a sedative effect lasting 8 h in the highest dose group (12 mg/kg).

3.2. Behavioral assessment

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

The effect of oxaliplatin treatment on *mechanical allodynia* is shown in Fig. 2A. In the 6 and 12 mg/kg dose groups, the withdrawal threshold decreased significantly compared with the control group on days 3 and 4 after injection in 100% of the rats ($p<0.05$ and 0.01, respectively), with a maximum reduction of 84% ($p<0.001$), which continued until day 8. The 3 mg/kg dose induced a significant decrease in the paw withdrawal thresholds from day 4 after injection ($p<0.01$).

Fig. 2B shows the effect on *mechanical hyperalgesia*. No significant difference was observed between all the treated groups and the control.

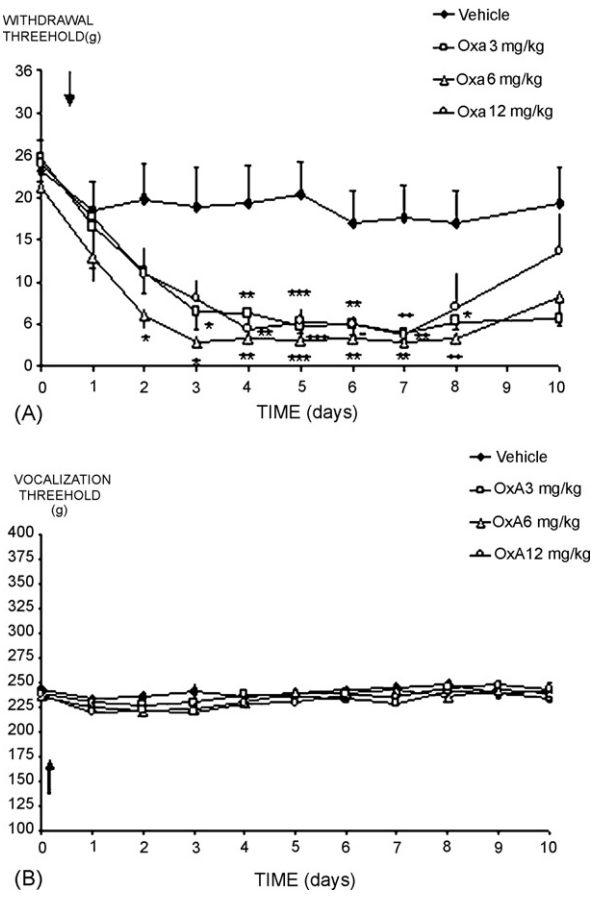

Fig. 2. Mechanical allodynia (A) and hyperalgesia (B). Paw withdrawal threshold of control (♦, vehicle, $n=8$) and treated (□, oxaliplatin 3.0 mg/kg, $n=8$); (△, oxaliplatin 6.0 mg/kg, $n=8$); (◯, oxaliplatin 12 mg/kg, $n=8$) rats to von Frey hairs and pressure applied to the plantar surface of the hindpaw. Each rat received a single intraperitoneal injection (↓). Results are expressed as mean ± S.E.M. 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 a Bonferroni *t*-test) in only mechanical allodynia (A) compared with the vehicle group was observed on the days 3 and 4 after injection for the 6 and 12 mg/kg dose groups and persisted until day 8.

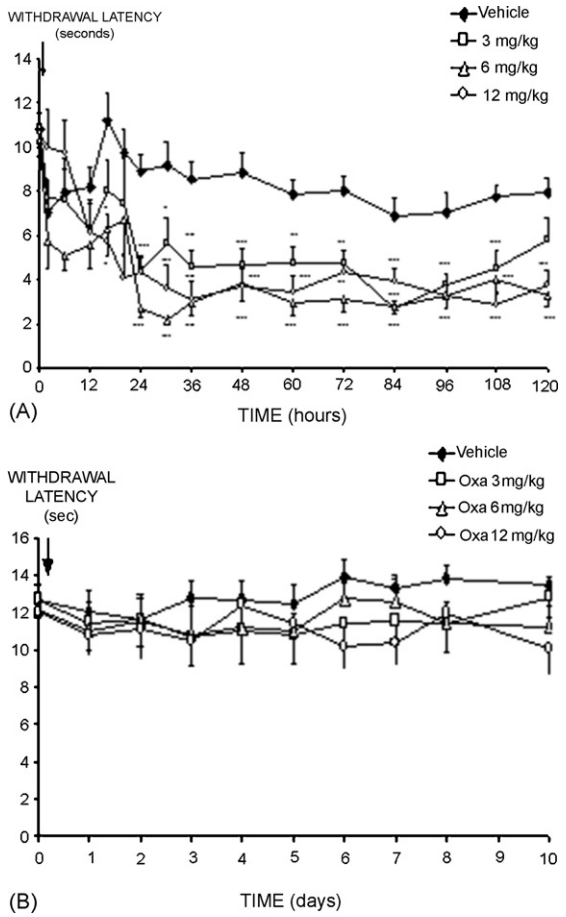

Fig. 3. Cold (A) and heat (B) thermal allodynia. The figure shows tail withdrawal latencies of control (♦, vehicle, $n=8$) and treated (□, oxaliplatin 3.0 mg/kg, $n=8$); (△, oxaliplatin 6.0 mg/kg, $n=8$); (○, oxaliplatin 12 mg/kg, $n=8$) rats after immersion of the tail in a cold (10 °C) or hot (42 °C) water bath. Each rat received a single intraperitoneal injection (↓). Results are expressed as mean ± S.E.M. No significant variation was obseved on control rats. A significant difference (*$p<0.05$, **$p<0.01$, ***$p<0.001$, analysis of variance followed by a Bonferroni t-test) in cold allodynia (A) compared with the vehicle group was observed from 24 h and remained low up to day 5 for the 6 and 12 mg/kg groups. No significant difference was observed in heat thermal allodynia in the tail withdrawal latency compared with the control whatever the injected dose.

Fig. 3A and B demonstrate the effect of oxaliplatin injection on *thermal allodynia* symptoms induced either by a non-noxious cold stimulus (10 °C) or a non-noxious hot stimulus (42 °C) (Necker and Hellon, 1978; Allchorne et al., 2005). In cold water (10 °C), the control group showed a non-significant reduction of withdrawal latencies for 12 h after the vehicle injection, completely recovering at 16 h until the end of the experiment. In the 3 mg/kg oxaliplatin group, a significant decrease was shown from 24 h after the injection until 84 h −58%, $p<0.001$). It then slowly reverted to control values (−34% at $t=120$ h, $p<0.05$). In the 6 mg/kg group, a strong significant decrease was observed from 24 to 36 h with a maximum reduction of −76% ($p<0.001$). Withdrawal latencies then remained persistently low up to 120 h (−49%, $p<0.001$). In the 12 mg/kg group, a significant decrease was observed from the 24 h ($p<0.001$), reaching a maximum reduction of −69% at $t=36$ h ($p<0.001$) and stable to day 5. In hot water (42 °C), no significant reduction in the tail withdrawal latency appeared compared with the control whatever the injected dose after exposure to a non-noxious heat stimulus.

Fig. 4A and B demonstrate the effect of oxaliplatin injection on *thermal hyperalgesia* symptoms induced either by a noxious cold stimulus (4 °C) or a noxious hot stimulus (46 °C), respectively. After use of a cold noxious stimulus (4 °C), a significantly reduction of tail withdrawal latency compared with controls was shown in 100% of the rats in the 6 mg/kg dose group from 26 h ($p<0.05$) after the oxaliplatin injection reaching a maximum reduction of 65% ($p<0.001$) at day 6 and incomplete recovery up to day 10. In the 3 and 12 mg/kg dose groups, a significant decrease appeared from day 4 ($p<0.01$) to day 6. In the 3 mg/kg group, the recovery was gradual during the last 4 days of the experiment whereas a total recovery was rapidly observed at day 7 in the 12 mg/kg group. Exposure to a heat noxious stimulus (46 °C) did not display a significant difference for any of the treated groups compared with controls.

3.3. Immunohistochemical study

Fig. 5 shows the immunoreactivity of CGRP in spinal cord. No difference was observed in the spinal dorsal horn between the vehicle and the oxaliplatin-treated group. The mean immunostaining was similar in control and treated rats (17%).

In Fig. 6, substance P immunoreactivity showed a significant difference in the spinal dorsal horn between the oxaliplatin-treated and the vehicle group (12% versus 4%, $p<0.05$), but it was similar between the two dorsal horns.

4. Discussion

The absence of any deterioration in the general state of the animals after the single administration of 3 or 6 mg/kg oxaliplatin enabled us to validate all these behavioral pain tests. No significant variation in body temperature was observed at the three different oxaliplatin doses. The intraperitoneal injection route was selected in reference to Cavaletti et al. (2001),

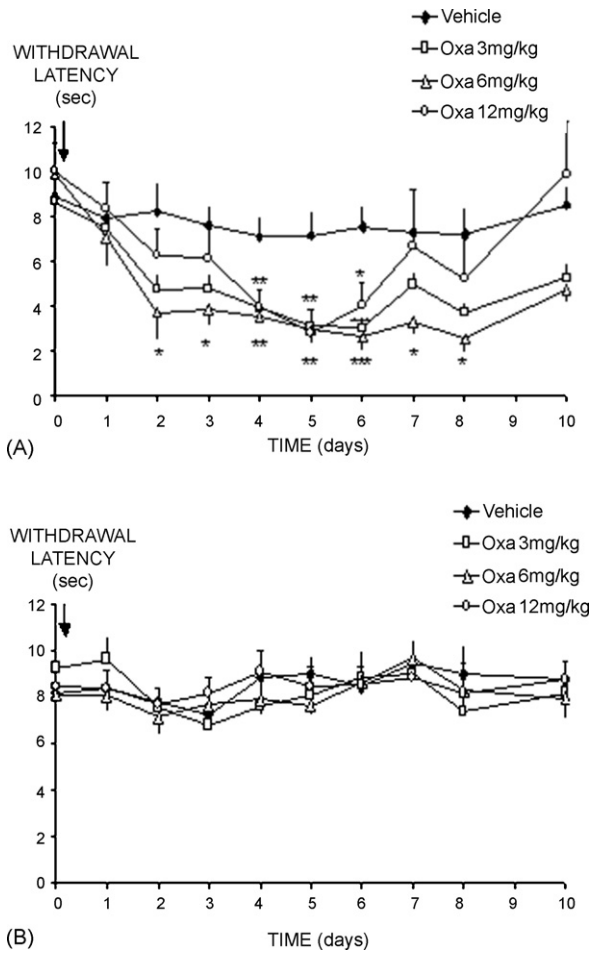

Fig. 4. Cold (A) and heat (B) thermal hyperalgesia. The figure shows tail withdrawal latencies of control (♦, vehicle, $n=8$) and treated (□, oxaliplatin 3.0 mg/kg, $n=8$); (△, oxaliplatin 6.0 mg/kg, $n=8$); (◯, oxaliplatin 12 mg/kg, $n=8$) rats after immersion of the tail in a cold (4 °C) and hot (46 °C) water bath. Each rat received a single intraperitoneal injection (↓). Results are expressed as mean ± S.E.M. 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) compared with the vehicle group was observed from the 26 h after single oxaliplatin injection in the 6 mg/kg dose group and from day 4 in the 3 and 12 mg/kg, gradual recovery at days 10 and 7, respectively. In contrast, no significant variation was observed in any dosed group compared with the control after a single oxaliplatin injection.

Holmes et al. (1998) and Jamieson et al. (2005). The 6 mg/kg dose was higher than that used by Cavaletti et al. (2001) who injected oxaliplatin intraperitoneally at 4 mg/kg dose for nine injections. In clinic trails, the anticancer treatment uses 85 mg/m^2 every 2 weeks or 130 mg/m^2 every 3 weeks, and the maximum tolerated dose is 200 mg/m^2, which corresponds approximately to 6 mg/kg dose (Raymond et al., 1998; Carrato et al., 2002). The highest dose (12 mg/kg) is close to the lethal dose 50 (14.3 mg/kg) after acute intraperitoneal injection in the rat, but no rat died in the course of the experiment, though rats remained sedated for at least 8 h after the treatment. Hence, we considered that this dose was not useful as it would influence subsequent responses to pain tests.

We describe, after a single oxaliplatin administration in rats, some significant behavioral nociceptive signs that closely mimic those observed in cancer patients. In humans, oxaliplatin induces undesirable neurological symptoms, making it sometimes necessary to stop the treatment, and in particular acute cold allodynia with paresthesia, hypoesthesia, dysesthesia of the hands, feet, peri-oral area or throat. Importantly, no relevant animal model for studying this specific painful neuropathy, which is a feature of current clinical practice, has been reported yet. Studies published to date on oxaliplatin chronic administration in rats have only reported histological or electrophysiological alterations or mechanical hyperalgesia (Holmes et al., 1998; Cavaletti et al., 2001, 2002; Jamieson et al., 2005; Ghirardi et al., 2005).

Our study reports major behavioral effects of oxaliplatin administration on nociceptive thresholds induced by either non-noxious or noxious thermal stimuli. Prompt cold allodynia was observed in all the dose groups, and remained low from 24 h to day 5 after a single oxaliplatin injection. The 3 and 6 mg/kg doses in rats gave stable low nociceptive thresholds between 24 and 120 h without clinical manifestations. The maximum intensity (−76% versus baseline) was observed at 36 h in the 6 mg/kg group and thresholds remained persistently low, up to 120 h. Similar low values were observed after 3 and 12 mg/kg, demonstrating the neurotoxic effect of all the oxaliplatin doses, even after a single intraperitoneal injection. On the other hand, a cold hyperalgesia was observed 26 h after a 6 mg/kg dose injection, reaching a maximum reduction of 65% at day 6, a slow recuperation for 3 and 6 mg/kg doses with a faster one for the 12 mg/kg dose. Heat thermal allodynia and hyperalgesia were not observed. These very specific signs can be paired with the fact that about 85 to 95% of oxaliplatin-treated patients rapidly develop significant neurological symptoms, such as cold-induced distal dysesthesia and (or) paresthesia during the infusion period, reaching a peak within the first 24–48 h (Extra et al., 1990; Mathe et al., 1986; Ibrahim et al., 2004; Cersosimo, 2005).

Significant decreases in mechanical allodynia were displayed in the 6 and 12 mg/kg dose groups on days 3 and 4 after the injection, with a maximum reduction (84%) at 6 mg/kg dose, whereas no mechanical hyperalgesia occurred. These behavioral effects are similar to those observed in humans. Acute dysesthesia were

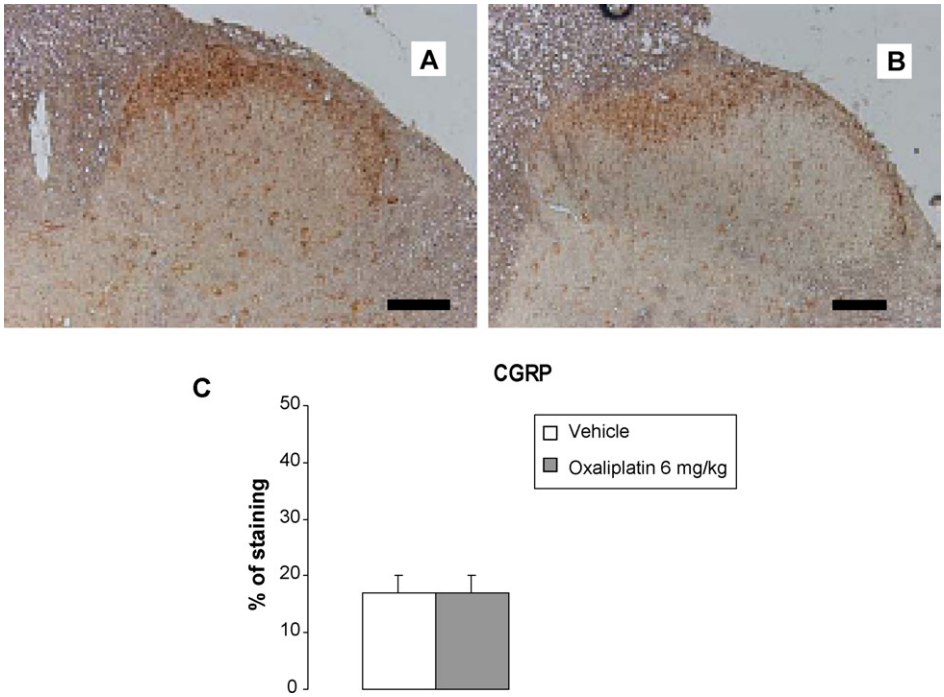

Fig. 5. Photomicrographs of CGRP immunostaining in the lumbar dorsal horn of the spinal cord 24 h after a single i.p. injection of vehicle (A) or oxaliplatin (B) (6.0 mg/kg, $n = 6$), scale bar = 50 μm. (C) Quantitative measurement of corresponding CGRP-immunoreactivity. No significant variation was observed between the vehicle and the 6 mg/kg dose group.

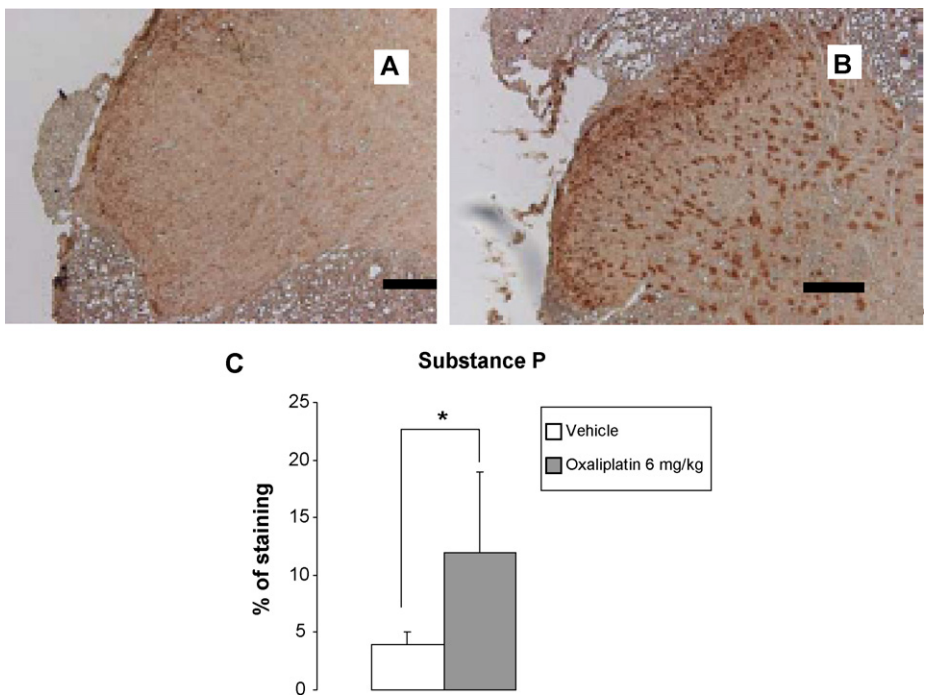

Fig. 6. Photomicrographs of substance P immunostaining in the lumbar dorsal horn of the spinal cord 24 h after a single i.p. injection of vehicle (A) or oxaliplatin (B) (6.0 mg/kg, $n = 6$), scale bar = 50 μm. (C) Quantitative measurement of corresponding substance P immunoreactivity. The mean number of pixels per area occupied by substance P immunoreactive cells in the dorsal horn of rats 24 h after a single oxaliplatin injection was significantly increased compared with their respective counterpart in the dorsal horn of vehicle-injected rat (*$p < 0.05$).

observed after oxaliplatin infusion (72% of patients and 87% of treatment cycles) (Soulie et al., 1997) with moderate symptoms appearing during or immediately after the infusion, lasting for a few min to a few days but sometimes recurring over the following week (Soulie et al., 1997; Raymond et al., 1998). These undesirable sensory effects seem to be concentration-related; it was shown that a lower peak plasma concentration of oxaliplatin could prevent dysesthesia (Gamelin et al., 2002; Grothey, 2005). Ghirardi et al. (2005) showed a mechanical hyperalgesia induced by oxaliplatin administered intraperitoneally at 3 mg/kg six times in rats; in our study a single intraperitoneal injection did not induce mechanical hyperalgesia.

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 chronically induced whereas neurotoxicity can be produced by either acute or repeated oxaliplatin injections. Also, oxaliplatin-induced cold allodynia and hyperalgesia signs appear more rapidly and are more completely reversible than cisplatin-induced signs. Comparison with other published single oxaliplatin doses is not possible as there is no literature on acute animal models of neuropathy induced by a single oxaliplatin administration in rats. When compared with previously published results, intensities of acutely induced cold hyperalgesia and allodynia signs are similar to those of the chronically induced signs (−65% versus −87% and −76% versus −85%, respectively) (Ling et al., 2007). Mechanical allodynia is of similar intensity (−84% versus −85%). However, unlike chronic administration, the acute oxaliplatin administration does not induce either heat thermal hyperalgesia and allodynia, or mechanical hyperalgesia. These different behavioral results suggest that the cellular and molecular targets may be linked to the mode of oxaliplatin administration (Jamieson et al., 2005).

Such brief toxic induction may cause minimal nerve lesions. The main presence of allodynia symptoms may mean that only the most sensitive fibers are concerned by this toxic aggression. One mechanism of oxaliplatin toxicity is the entry of oxalate into the cell, chelation of Ca^{++} ions and alteration of voltage-dependent sodium channels in some sensory nerves, resulting in a hyperexcitable state (Grolleau et al., 2001). Also, the facts that (i) oxaliplatin acts more on the A-fibers than the C-fibers (Adelsberger et al., 2000), (ii) oxaliplatin did not modify responses to heat or mechanical noxious stimuli, mainly transported by unmyelinated C axons, (iii) mechanical allodynia is mediated by A-type low-threshold mechanoreceptors, suggest the implication of myelinated, fast-conducting small-diameter A-fibers in cold allodynia. In opposite McKemy et al. (2002) previously showed that cold-sensitive fibers, nevertheless in the wide temperature range 8–28 °C, were C-fibers.

CRRP and substance P are excitatory neurotransmitters and (or) neuromodulators that are released in the spinal dorsal horn by the primary sensory afferents, thus contributing to the development of allodynia and hyperalgesia by facilitating the release of excitatory glutamate and aspartate from primary afferents (Ma and Eisenach, 2003). Whereas substance P is restricted to A- and C-fiber nociceptors, the absence of CGRP immunoreactivity in spinal cord may be linked to the absence of alteration of C-fibers. In addition, the amount of SP immunoreactivity is higher than for CGRP because the single oxaliplatin injection corresponds to a temporally limited stimulation of A-fibers in the first phase of the evolution. In this phase, the nociception pathways using SP are more briefly induced, CGRP being released only in the pain maintenance phase (Afrah et al., 2002; Jang et al., 2004; Pitcher and Henry, 2004).

This study reproduces, for the first time in animals, the neurotoxic profile of a single oxaliplatin administration, characterized by a rapid onset and a high intensity of sensory disturbances, especially hypersensitivity to cold with allodynia and hyperalgesia, suggesting possible functional alteration of myelinated sensory afferent fibers with involvement of substance P. These selective effects may clue up the mechanistic basis for the acute oxaliplatin neuropathy leading to a better understanding of the clinical condition and to optimize its treatment.

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