Comparative antiallodynic activity of morphine, pregabalin and lidocaine in a rat model of neuropathic pain produced by one oxaliplatin injection

Bing Ling a,b, François Coudoré c,*, Loic Decalonne a,b, Alain Eschalier a,d, Nicolas Authier a,b

a INSERM, U766, Facultés de Médecine et de Pharmacie, 63001 Clermont-Ferrand, France
b Université Clermont 1, Faculté de Pharmacie, Laboratoire de Toxicologie, 63001 Clermont-Ferrand, France
c Université Paris-Sud, EA3544, Faculté de Pharmacie, Laboratoire de Neuropharmacologie, 92296 Chatenay-Malabry, France
d Université Clermont 1, Faculté de Médecine, Laboratoire de Pharmacologie Médicale, 63001 Clermont-Ferrand, France

A B S T R A C T

A single infusion of oxaliplatin, a drug active against colorectal cancer, induces specific painful syndrome characterized by neurosensitive symptoms triggered or aggravated in cold conditions. In an animal model that reproduces such hypersensitivity to cold for five days after a single oxaliplatin administration (6 mg/kg, i.p.), we assessed the antinociceptive efficacy of intravenously administered drugs such as morphine, lidocaine and pregabalin using the rat tail immersion test in cold water (10 °C). The antinociceptive efficacy was first ranked by ratio of the pharmacological effect (versus time) to dose: pregabalin (2 mg/kg) > lidocaine (3 mg/kg) > morphine (4 mg/kg). Our results show that pregabalin may be a good choice to treat cold hypersensitivity after one oxaliplatin injection.

1. Introduction

Oxaliplatin is a platinum-based chemotherapeutic agent used in the treatment of advanced metastatic colorectal cancer (Baker, 2003). In clinical use, a single oxaliplatin infusion induces specific sensory neurotoxic signs triggered or aggravated by exposure to cold. This drug-induced toxicity is reported to occur in 85–95% of patients, especially during infusion, and peaks in the first 24–48 h (Grouleau et al., 2001). It is characterized by the rapid onset of cold-induced distal dysesthesia and/or paresthesia, muscle tightness in the throat and jaw, tetanic spasm, myotonia, and prolonged muscular fasciculation of legs, hands and jaws (Gamelin et al., 2002; Lehky et al., 2004). The symptoms disappear in about a week but recur on subsequent infusion. The neurotoxic profile of oxaliplatin is specific; the other platinum-based chemotherapeutic agents also cause a sensory neuropathy after chronic treatment, but do not produce such acute painful symptoms after only one administration.

There are few reports on the use of drugs to prevent or treat these painful symptoms of oxaliplatin-induced acute syndrome in clinical practice (Cersosimo, 2005). Numerous drugs have been proposed in clinical practice to treat chronic drug-induced neurotoxicity (Carrato et al., 2002; Gamelin et al., 2004) and also calcium or magnesium (Cersosimo, 2005; Grothey, 2005; Durand et al., 2005). No precise pharmacological targets have been found and there is no clear strategy for preventing or attenuating the painful neurotoxicity observed after a single oxaliplatin administration.

In this work, we used an animal model that reproduces nociceptive signs similar to those presented by patients after one oxaliplatin administration (Ling et al., 2007). As in clinical practice, significant hypersensitivity symptoms to cold stimuli were promptly observed from 24 h to Day 5 with a maximum effect of 76% at 30 h. We assessed the pharmacological efficacy in this animal model of acute cold hypersensitivity of different antinociceptive drugs that could be used to treat this painful neuropathy in patients.

2. Materials and methods

2.1. 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. Drugs

Oxaliplatin was generously provided by Debiopharm (Lausanne, Switzerland). It was dissolved in a 5% glucose solution at a concentration of 1 mg/ml based on animal weight to ensure intraperitoneal injections of less than 2.5 ml. Oxaliplatin was intraperitoneally administered alone at 6 mg/kg. Volumes of a 5% glucose solution were adjusted to the weight of each rat and injected by the same route in the control group. All the tested drugs were obtained from Sigma (St-Quentin-Fallavier, France) or donated by the respective manufacturer.

2.3. Behavioral testing

Behavioral tests were conducted before 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 the tests were performed before oxaliplatin administration to assess baselines.

Hypersensitivity to cold was assessed using the tail immersion test in water maintained at 10 °C (Necker and Hellion, 1978; Allchorne et al., 2005). Half of the tail was immersed in water and maintained until tail withdrawal. 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.

2.4. Pharmacological protocols

Rats were habituated to handling by the investigator during the week before the experiment. Experiments were performed blindly in a quiet room by a single experimenter using the method of equal blocks (n = 8 rats per block) with randomization of treatments. Only animals presenting characteristic hypersensitivity to cold and a good clinical status were used to test the antinociceptive effects of various drugs. Tail withdrawal latencies were recorded until the threshold values in rats with oxaliplatin-induced hypersensitivity had reverted to pre-drug levels for at least one drug dose.

Drug administration:

- Morphine hydrochloride (Cooperation 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 into the tail vein.

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

- Pregabalin (Pfizer, Paris, France) was dissolved in 0.9% NaCl just before the intravenous administration at 2, 10, or 100 mg/kg dose.

2.5. Statistical analysis

The time course of the pharmacological effect was examined using analysis of variance (ANOVA) followed by a Bonferroni t-test when the F value was significant (Statview 4.55, Abacasis Concept Inc., Berkeley, CA, USA). The mean area of the tail withdrawal latency was compared using one-way analysis of variance followed by a Bonferroni t-test to detect differences between each treatment and the control group. Data were expressed as means ± 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.

3. Results

No rat displayed any loss of weight or alteration in clinical signs. Clinical status remained good for all the groups. No piloerection, eye closing, diarrhoea or specific postures were observed.

For hypersensitivity to cold water (+10 °C) (Fig. 1), the treated groups showed a nonsignificant reduction of withdrawal latencies of the tail on Day 1 after the injection compared with the vehicle. In the 6 mg/kg group, a significant reduction was observed on Day 2 with a maximum reduction of ~76% (t = 30 h, P < 0.001). Values remained low until Day 6 (~49%, P < 0.001).

All the morphine doses induced significant dose-related antiallodynic effects between 15 and 60 min post-injection (Fig. 2 A, B). The strongest effect occurred 15 min after the 4 mg/kg dose (+368%, P < 0.001). The 1 and 2 mg/kg doses induced an increase in tail withdrawal latency but of lower intensity (+204%, +266%, P < 0.05, P < 0.01, respectively). The areas under the curve (AUCs) showed a significant increase for the 2 mg/kg group (P < 0.01, +130%) and the 4 mg/kg group (P < 0.001, +129%) in comparison with saline. The 2 mg/kg response was more prolonged than that of the other doses after 120 min.

Only the 3 mg/kg i.v. lidocaine dose induced a significant antiallodynic effect between 75 and 150 min (+133%, P < 0.01) after the injection (Fig. 3 A, B) with a maximal effect at the 90th minute postadministration (+257%, P < 0.001). The AUCs of the 1 and 6 mg/kg doses were not significantly different from the vehicle.

After the lowest dose of pregabalin (Fig. 4 A, B), a significant antiallodynic effect was observed with a maximum effect at 45 min (+187%, P < 0.05). By contrast, the 10 and 100 mg/kg doses did not induce a significant effect. The AUC values confirmed a significant difference from control only for the 2 mg/kg dose (+124%, P < 0.05).

4. Discussion

We found major behavioral effects of oxaliplatin administration on nociceptive thresholds induced by a cold stimulus. The oral oxaliplatin model of chemotherapy-induced neuropathic pain, originally developed by Ling et al. (2007), has proved a reliable tool for the study of thermal hypersensitivity. Cold hypersensitivity remained low from Day 1 to Day 5 after oxaliplatin injection. The 6 mg/kg dose gave stable low nociceptive thresholds without clinical manifestations. The maximum intensity (~76% versus baseline) was observed at 30 h in the 6 mg/kg group and thresholds remained persistently low up to Day 5, demonstrating the neurotoxic effect of this oxaliplatin dose, even after a single intraperitoneal injection. These very specific signs can be paired with the fact that about 85–95% 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 (Cersosimo, 2005). 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). The mechanisms of the sensitivity to cold exposure observed with oxaliplatin are not well understood. There is no literature on the neuronal consequences of transient oxaliplatin administration. At present we do not know whether oxaliplatin induces a cellular
stress through a state of nerve hyperexcitability, or whether cellular stress is a consequence of some other mechanism of damage in cell bodies and nerve fibers.

Using an animal model that reproduces symptoms of cold allodynia, we assessed the pharmacological efficacy of drugs that could be used to treat this severe painful neuropathy in humans, such as morphine, lidocaine or pregabalin. The behavioral test used in this work, the tail immersion test in water maintained at 10 °C, reflects allodynia, as the nonpainful temperatures in the naive rats range from 10 to 15 °C (Allchorne et al., 2005).

Morphine and lidocaine doses were similar to those used in previous work by Ling et al. (2007) in the animal pain model obtained with chronically administered oxaliplatin. Pregabalin doses were determined from Nozaki-Taguchi et al. (2001) (80 mg/kg, i.p.), Beyreuther et al. (2006) (100 mg/kg, i.p.), Takeuchi et al. (2007) (10 and 30 mg/kg, i.p.) and Han et al. (2007) (3–30 mg/kg, i.p.). No adverse effect of these drug doses was observed by visual examination during either experimentation. To compare the antinociceptive effect between drugs, values were calculated taking into account the AUC of the corresponding vehicle, the duration of the experiment and the drug dose. We classified all the tested drugs using the ratio (AUC of drug effect – AUC of vehicle effect) to dose. Comparison of the effects gave the following result: pregabalin (2 mg/kg i.v.) > lidocaine (3 mg/kg, i.v.) > morphine (4 mg/kg, i.v.).

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**Fig. 2.** (A and B) Morphine administration. Time course (A) and area under the curve versus time (B) of the effect of a single intravenous injection of morphine (■, morphine 1 mg/kg; △, morphine 2 mg/kg; ○, morphine 4 mg/kg) or saline (●) on tail withdrawal latencies using the tail immersion test in cold non-noxious (10 °C) water in a rat model of neuropathy induced by a single oxaliplatin injection (6 mg/kg, i.p.). Arrow corresponds to injection. Values are determined before and after induction of the neuropathy and every 15 min for 120 min after morphine injection. Results are expressed in seconds as means ± S.E.M; n = 8 in each group. *P < 0.05, **P < 0.01, ***P < 0.001, versus the corresponding pre-drug values (ANOVA followed by Bonferroni t-test). There were no significant variations in control rats. The mean area of the tail withdrawal latency versus time curve (AUC) was calculated with the trapezoidal rule and compared using one-way analysis of variance (ANOVA) followed by a Bonferroni t-test to detect differences among each treatment and the control group.

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**Fig. 3.** (A and B) Lidocaine administration. Time course (A) and area under the curve versus time (B) of the effect of a single intravenous injection of lidocaine (□, lidocaine 1 mg/kg; △, lidocaine 3 mg/kg; ○, lidocaine 6 mg/kg) or saline (●) on tail withdrawal latencies using the tail immersion test in cold non-noxious (10 °C) water in a rat model of neuropathy induced by a single oxaliplatin injection (6 mg/kg, i.p.). Arrow corresponds to injection. Values are determined before and after induction of the neuropathy and every 15 min for 240 min after lidocaine injection. Results are expressed in seconds as means ± S.E.M; n = 8 in each group. *P < 0.05, **P < 0.01, ***P < 0.001, versus the corresponding pre-drug values (ANOVA followed by Bonferroni t-test). There were no significant variations in control rats. The mean area of the tail withdrawal latency versus time curve (AUC) was calculated with the trapezoidal rule and compared using one-way analysis of variance (ANOVA) followed by a Bonferroni t-test to detect differences among each treatment and the control group.
Pregabalin, a structural analog of gabapentin, is used in the treatment of pain in diabetic and post-herpetic peripheral neuropathy (Dworkin and Kirkpatrick, 2005). It is a selective, high-affinity ligand for the α2-δ protein subunit of voltage-dependent calcium channel and modulates the release of neurotransmitters resulting in analgesic effects on neuropathic pain. Pregabalin reduces calcium influx and diminishes release of several excitatory neurotransmitters, including glutamate, noradrenalin and substance P, thus interfering with nociceptive signal transfer. We observed that the effect of the low pregabalin dose (2 mg/kg) was longer and more stable than that of the other doses after 120 min, which could explain the higher AUC of this pregabalin dose. It has been suggested that pregabalin exerts its antiallodynic effect mainly on the spinal cord (Han et al., 2007). A kinetic explanation of such a maintained effect may be that more gradual and persistent levels of pregabalin in spinal cord are obtained after the low dose of 2 mg/kg, which may saturate sodium channels, the site of action, better than the 10 or 100 mg/kg doses. It has been recently suggested that the antiallodynic effect of pregabalin is mainly mediated by a spinal mechanism, although supraspinal or peripheral mechanisms cannot be excluded. Only intrathecally administered pregabalin dose dependently attenuated cold allodynia with the acetone test in neuropathic pain models induced by either ligation of the L5 and L6 spinal nerves or by transection of the tibial and sural nerves (Han et al., 2007).

During the first 30 min, intravenously administered morphine was the most efficient drug. The efficacy of morphine has not been clearly established in painful neuropathy. There is controversy about the relative efficacy of opiate analgesics against neuropathic pain. In the CCI model, an i.v. morphine dose of 1 mg/kg resulted in an antinociceptive effect in mechanical tests, while higher doses caused sedation (Kayser et al., 1993). In a model where the neuropathy was produced by chronic constriction injury of the infraorbital branch of the trigeminal nerve, the mechanical allodynia-like symptoms were resistant to i.v. morphine (Idanpaan-Heikkila and Gutthaud, 1999). However, the potency of systemic administered morphine was clearly reported in rat models of peripheral nerve injury (Erichsen et al., 2004). In the spinal nerve ligation model of neuropathic pain, subcutaneous administration of morphine (5 mg/kg) had anti- nalodynic effects in a test of cold allodynia induced by a drop of acetone on the plantar surface (Lemberg et al., 2006). Compared with pregabalin and lidocaine, morphine efficacy probably involves other mechanisms of action. It has been shown that morphine is more efficient on nociception mediated by C-fibers than A-fibers but morphine may also potentiate the inhibitory supraspinal influences on the nerve hyperactivity (Le Bars et al., 2001).

The observed antiallodynic effect of lidocaine was less marked. The lower effect of the higher dose of 6 mg/kg observed in the U-shape dose response may be explained by a cardiac effect with a bradycardia and a tendency to lower spontaneous activity and motricity. Previous personal results gave a cardiac mortality with a 9 mg/kg dose, explaining why the 6 mg/kg and higher lidocaine doses may be toxic. Much of the injury-induced remodelling of sensory neurone function associated with nociceptive response is linked to a regulation of voltage-activated Na⁺ channel expression within dorsal root ganglion neurons. Thus, the peripheral mechanism of allodynia may be mediated by a sodium channel that is blocked by low concentrations of local anesthetics. A suppression of responses to cold allodynia at 10 °C by systemic lidocaine (0.6–1.8 mg/kg, i.v.) has been reported in a CCI model (Jasmin et al., 1998). Marked local changes in the activity of sodium channels could explain the low responses to cold stimuli, although lidocaine does not cross the blood–nerve barrier, normally leaky in the dorsal root ganglion. Also, it is reported that lidocaine (up to 40 mg/kg, i.p.) had no effect on cold allodynia in two rat models of neuropathic pain, the photochemically induced nerve injury model and spared nerve injury model, but it seems that the modalities of lidocaine administration and consequently the lidocaine local plasma levels obtained modulate the antinociceptive activity (Erichsen et al., 2003).

To date, only a few publications show the antinociceptive effects of these drugs in chemotherapy-induced animal pain models. Using vincristine-induced neuropathic rats subjected to the mechanical allodynia test, Nozaki-Taguchi et al. (2001) showed a significant reversal of allodynia after intraperitoneal injection of 5 mg/kg morphine, 45 mg/kg lidocaine, or 80 mg/kg pregabalin, Linch et al. (2004) observed an ED50 of 0.62 μmole/kg i.p. morphine, and Rahn et al. (2007) observed an effect of 8 mg/kg i.p. morphine dose on
mechanical allodynia. Using paclitaxel-induced neuropathic rats, Flatters and Bennett (2004) concluded that there was a resistance of this model to morphine as they did not observe any effect of a 4 mg/kg morphine dose on mechanical allodynia/hyperalgesia but a 50% reversal of the 8 mg/kg dose, without examining a possible sedation from this last dose.

To conclude, these data obtained in the rat oxaliplatin-induced neuropathic pain model point to the utility of intravenously injected pregabalin as a good choice to treat the neuropathic symptoms, especially the very unpleasant hypersensitivity to cold when oxaliplatin is acutely administered. This finding underlines the important role of sodium channels in the symptoms observed after a single oxaliplatin infusion.

References


