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Pharmacological comparison of a nonhuman primate and a rat model of oxaliplatin-induced neuropathic cold hypersensitivity

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Abstract

Oxaliplatin is a first-line treatment for colorectal cancer. However, shortly following treatment, cold-evoked hypersensitivity appears in the extremities and over time, the pain is such that oxaliplatin dosing may need to be markedly reduced or even terminated. There is currently a lack of efficacious treatments for oxaliplatin-induced peripheral neuropathy, which is due in part to the difficulty in translating findings obtained from preclinical rodent models of chemotherapy-induced peripheral neuropathy. Nonhuman primates (NHP) are phylogenetically closer to humans than rodents and may show drug responses that parallel those of humans. A significant decrease in tail withdrawal latency to 10°C water ("cold hypersensitivity") was observed beginning 3 days after intravenous infusion of oxaliplatin (5 mg/kg) in *Macaca fascicularis*. A single treatment of duloxetine (30 mg/kg, p.o.) ameliorated oxaliplatin-induced cold hypersensitivity, whereas pregabalin (30 mg/kg, p.o.) and tramadol (30 mg/kg, p.o.) did not. By contrast, in rats, no significant cold hypersensitivity, or increased responsiveness to acetone applied to the hind paws, was observed 3 days after the first injection of oxaliplatin (5 mg/kg, i.p., once per day, two injections). Therefore, rats were tested after six treatments of oxaliplatin, 17 days after the first treatment. All analgesics (30 mg/kg, p.o.) significantly ameliorated cold hypersensitivity in rats. The activity of analgesics in the oxaliplatin-treated macaques parallel clinical findings. The current results indicate that the NHP could serve as a bridge species to improve translatability of preclinical findings into clinically useful treatments for oxaliplatin-induced peripheral neuropathy.

Abbreviations

BUN, blood urea nitrogen; CIPN, chemotherapy-induced peripheral neuropathy; NHP, nonhuman primate.

Introduction

A common adverse effect across many classes of anticancer drugs is the development of a painful peripheral neuropathy (Miltenburg and Boogerd 2014). In general, 30–40% of chemotherapy patients develop a painful peripheral neuropathy, though the incidence and severity greatly differs between drugs (Wolf et al. 2008; Hershman et al. 2014). The symptoms are reminiscent of other painful peripheral neuropathies, such as painful diabetic neuropathy, in which a symmetrical dysesthesia and paresthesia emerges distally in the fingers and toes, in a "glove and stocking" distribution. Chemotherapy-induced peripheral neuropathy (CIPN) appears to be dose dependent, in that symptom severity increases with dose, leading to dose reduction or delayed treatment, greatly constraining the efficacy of chemotherapeutics. Thus, there is a need for effective analgesics that ameliorate CIPN so that patients receive the benefit of a complete course of chemotherapy.

Oxaliplatin, a platinum-based chemotherapeutic, is the standard treatment for advanced colorectal cancer. Unique to oxaliplatin is the onset of an acute or early onset neuropathy that is aggravated by cold temperatures. Virtually all patients report this neuropathy, which emerges within hours or days following treatment. The acute symptoms are transient in nature, persisting up to 7 days following treatment (Attal et al. 2009; Grisold et al. 2012; Miltenburg and Boogerd 2014).

Although there are a number of drugs approved for the management of neuropathic pain, there are currently no analgesics approved specifically for use in CIPN (Hershman et al. 2014). Preclinical studies utilizing rodent models of oxaliplatin-induced peripheral neuropathy suggest a variety of mechanism-based treatments for clinical oxaliplatin-induced neuropathic pain such as the anticonvulsant gabapentin (Authier et al. 2009). However, gabapentin, either in tandem with oxaliplatin treatment or during established neuropathy, failed to demonstrate efficacy in clinical trials (Mitchell et al. 2006; Rao et al. 2007).

One potential preclinical hurdle in the discovery of new analgesics for CIPN is the overreliance on a model species that is phylogenetically distant from humans (Kumar and Hedges 1998). Significant biological differences between rodents and humans could lead to erroneous predictions of the activity of potential therapeutics (Fitzgerald 2009; Huggins et al. 2012; Chen et al. 2013). Nonhuman primates (NHP) are a favored species in neuroscience research because of their complex neuroanatomy, compared to that of rodents, and their expression of clinically relevant symptoms in neurological disorders such as in Parkinson's disease (Courtine et al. 2007; Capitanio and Emborg 2008).

In the current study, a NHP model of oxaliplatin-induced peripheral neuropathy was developed and frequently prescribed analgesics for neuropathic pain were tested on oxaliplatin-induced cold hypersensitivity. In parallel, the analgesics were tested in a rodent model of oxaliplatin-induced peripheral neuropathy, as a "reference model."

Materials and Methods

Animals

Female cynomolgus macaques (*Macaca fascicularis*) were purchased from Shin Nippon Biochemical Laboratories, Ltd. (Kagoshima, Japan) and were 2.5–4.6 kg at the beginning of the study. Macaques were housed in individual stainless steel cages in a dedicated primate unit. Although individually housed, visual and auditory contacts were maintained between macaques and environmental enrichment was available in each cage. Macaques had free access to tap water and were fed standard NHP chow (Oriental Yeast Co., Ltd., Chiba, Tokyo, Japan), which was supplemented weekly with fresh fruits or vegetables. Male Sprague Dawley rats weighing 240–250 g were purchased from Charles River Japan (Kanagawa, Japan). Rats were housed in groups of four per cage, with light from 07:00 to 19:00 h. Rats had free access to food and water. Conditions of the holding rooms and behavioral testing rooms were according to standards described in the Guide for the Care and Use of Laboratory Animals, Eighth Edition (National Research Council).

All study procedures were reviewed and approved by the Hamamatsu Pharma Research, Inc., Animal Care and Use Committee. The facilities where the studies were conducted are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Drugs

Oxaliplatin and the μ-opioid/noradrenergic and serotonergic uptake inhibitor analgesic tramadol HCl were obtained from Sigma-Aldrich Japan, Co. (Tokyo, Japan). The anticonvulsant pregabalin was obtained from Kemprotec, Ltd. (Cumbria, UK). The serotonergic–noradrenergic reuptake inhibitor duloxetine HCl was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Tramadol, pregabalin, and duloxetine were dissolved in distilled water (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) on the day of testing.

Oxaliplatin-induced peripheral neuropathy in NHP

Oxaliplatin was dissolved in 5% glucose/water solution (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) and intravenously (i.v.) infused over a period of 2 h in accordance with instructions for clinical administration (Eloxatin®, package insert, Bridgewater, NJ, Sanofi-Aventis). The doses of oxaliplatin used were 2.3 mg/kg, 3.5 mg/kg, and 5 mg/kg; (n = 6/dose). Control animals (n = 3) were i.v. infused with an equivalent volume of vehicle (5% glucose/water). To keep macaques that received 5 mg/kg oxaliplatin hydrated, animals were subcutaneous treated once a day for 7 days with Ringer's lactate solution (Terumo Co., Tokyo, Japan).

To examine the behavioral effect of repeated oxaliplatin treatment over time, macaques underwent three treatment cycles with either vehicle (n = 3) or 5 mg/kg oxaliplatin (n = 5–6). Tail withdrawal latencies were measured before i.v. infusion and 1, 3, 5, and 7 days after each infusion. Additional withdrawal latencies were measured 14, 17, 19, and 21 days after the third infusion.

The tail immersion test in NHP

Macaques were habituated to restraint in a monkey chair and to the testing procedures by the investigators for about 2 h a day, 5 days a week, for at least 2 weeks before the start of the experiments. The behavioral assay used in the current study is a modification of the NHP tail withdrawal test (Dykstra and Woods 1986). Previously habituated macaques were restrained in a monkey chair. Hair from the distal 10 cm of the tail was removed with a hair clipper. Tail withdrawal latencies were timed with a stop-watch in 1 sec increments, up to a maximum (cut-off) latency of 20 sec. If an animal does not remove its tail from the water by 20 sec, the tail was removed from the water, and the cut-off latency was assigned. Three latencies were obtained, about 1 min apart, and the average was calculated. The distal tail was immersed in low (10°C), room (20°C), or warm (42°C) temperatures in random order. Fifteen minutes separated testing between temperatures.

While 20°C and 42°C can be considered non-noxious in the NHP, it is possible that 10°C is somewhat noxious (Dykstra and Woods 1986; Marchand et al. 1989; Butelman et al. 2003). However, prior to oxaliplatin or vehicle infusion, animals were able to keep their tails in 10°C for at least 20 sec. In addition, the water temperatures were not particularly noxious to the experimenters (Sukhtankar et al. 2014).

To test the effect of clinical analgesics on acute cold hypersensitivity, macaques were tested 3 days after either the first or second infusion of oxaliplatin, when maximal cold hypersensitivity was observed. Baseline withdrawal latencies to 10°C were obtained. Following baseline determination, the animals were randomized according to their withdrawal latencies and received 30 mg/kg (in 1 mL/kg, p.o.) of either duloxetine ($n = 4$), pregabalin ($n = 4$), or tramadol ($n = 3$). Animals were tested 1 h following dosing. Animals that received tramadol were tested 1 and 2 h after dosing.

The dose of the drugs (30 mg/kg) was in part extrapolated from the clinical doses and scaled to NHP (Food and Drug Administration 2005; Finnerup et al. 2015). No sedation was reported with any of the drugs at the currently tested dose.

Oxaliplatin-induced acute peripheral neuropathy in rats

The induction of oxaliplatin-induced peripheral neuropathy in rats was based on a previously published study (Sakurai et al. 2009). Oxaliplatin (5 mg/kg) was dissolved in 5% glucose/water solution and intraperitoneally (i.p.) injected in rats twice, following baseline behavioral testing and 1 day later (i.e., days 0 and 1). "Sham" rats were i.p. injected with an equivalent volume (2.5 mL/kg) of vehicle on the same schedule. A total of 79 rats were injected with oxaliplatin and eight rats were injected with vehicle ("sham"). Hind paw sensitivity to acetone (see below) was assessed in these rats 3 days after the first injection.

Because there was a lack of cold hypersensitivity 3 days following two oxaliplatin treatments (see Results), it was not possible to assess drug efficacy on early onset cold hypersensitivity. Thus, a separate group of rats was treated six times, twice weekly (days 0, 1, 7, 8, 14, and 15) for 3 weeks. A total of 80 rats were injected with oxaliplatin and 10 rats were injected with vehicle.

Following six treatments of oxaliplatin, rats were tested 16 days after the first injection (i.e., 1 day after the last injection of oxaliplatin) for baseline responses to acetone. On the following day, 17 days after the first oxaliplatin injection, rats were randomized and received 30 mg/kg (p.o.) of either duloxetine, tramadol, pregabalin, or an equivalent volume of vehicle (10 mL/kg). The drug doses were based on previous studies in rat pain models (Field et al. 1999; Combe et al. 2004; Iyengar et al. 2004).

The acetone test in rats

The acetone test was performed according to a method described previously (Flatters and Bennett 2004). Rats were placed in a plastic box (23 × 13 × 20 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. One hundred μL of acetone (Wako Pure Chemical, Ltd., Osaka, Japan) was applied to the plantar skin of each hind paw with a disposable 1 mL syringe with a 29-g needle (Terumo Co., Tokyo, Japan) and the hind paw response was observed for 20 sec following application of acetone. If there was no hind paw response to acetone during this period, then a score of 0 was assigned. However, if a hind paw response (flinch or stamping of the hind paw) occurred during the 20-sec period, then the rat's response was assessed for an additional 20 sec, for a total of 40 sec. A 4-point scale was used to grade the rat's response to acetone: 0, no response; 1, quick withdrawal, flick, or stamp of the paw; 2, prolonged withdrawal or repeated flicking (2≥) of the paw; 3, repeated flicking of the paw with licking directed at the ventral side of the paw (Flatters and Bennett 2004). A total of two applications were applied to each hind paw and a "cold score" was calculated by adding the scores from the four trials from each rat. The minimum score was 0 (a lack of response) and the maximum possible score was 12 (repeated hind paw licking and flinching following each application of acetone).

Blood chemistry

Platinum-based compounds are nephrotoxic. While oxaliplatin is less nephrotoxic than cisplatin, the nephrotoxicity in the macaque is not known (Kruger et al. 2015). Thus, to determine if oxaliplatin (5 mg/kg) leads to kidney dysfunction, blood samples (0.5 mL from the cubital vein) were obtained from macaques 3 days after the first oxaliplatin treatment. Samples were processed and analyzed for blood urea nitrogen (BUN) and creatinine (SP-4430; ARKRAY Infinity, Co., Kyoto, Japan).

Compliance with design and statistical analysis requirements

Rat

Each treatment group had at least 8–10 animals per group. Following oxaliplatin treatment, rats must have a cold score of ≥4 to be included in the drug efficacy experiments.

Nonhuman primate

A striking hypersensitivity to cold emerged 3 days after each oxaliplatin treatment. To take advantage of this early onset cold hypersensitivity, macaques were tested 3 days following the first oxaliplatin injection then again following the second oxaliplatin injection. Thus, the fewest number of NHP were used (Dykstra and Woods 1986; Sukhtankar et al. 2014). Less than five animals were used in the following experiments: three animals were treated with vehicle and tail sensitivity was evaluated over time (Fig. 3); four oxaliplatin-injected animals were dosed with duloxetine, four oxaliplatin-injected animals were dosed with pregabalin, and three oxaliplatin-injected animals were dosed with tramadol (Fig. 4). Macaques with withdrawal latencies of less than 10 sec (50% of cut-off) were included in the pharmacological studies. Animal testing was performed under blinded conditions.

Statistical analysis

Values are expressed as mean ± SEM. Randomization was performed using SAS Analytics Pro version 9.3 (SAS Institute, Tokyo, Japan) and EXSUS version 8.0a (CAC EXICARE Corporation, Osaka, Japan). Changes over time in the oxaliplatin-treated macaques were analyzed using a one-way repeated measures analysis of variance followed by Dunnett's test for post hoc comparisons (Graphpad Prizm 6, San Diego, CA). For statistical analysis, differences were considered significant at $P < 0.05$.

Results

Health status of oxaliplatin-treated NHP

Three days after macaques were treated with 5 mg/kg oxaliplatin, BUN was 16.0 ± 2.0 mg/dL and creatinine was 0.6 ± 0.1 mg/dL. In vehicle-treated macaques, BUN and creatinine were 17.7 ± 0.7 mg/dL and 0.9 ± 0.0 mg/dL, respectively. Blood urea nitrogen and creatinine levels in oxaliplatin-treated macaques did not suggest kidney dysfunction.

By the end of the 49-day observation period, a slight decrease in body weight was observed over time in oxaliplatin-treated macaques (2.8 ± 0.1 kg), but this was not statistically significant when compared with pretreatment body weight (3.2 ± 0.1 kg; $P > 0.05$). Both BUN and creatinine in these animals at the end of the observation period were within physiological limits.

Dose-dependent acute cold hypersensitivity in NHP following oxaliplatin treatment

A dose-dependent reduction in tail withdrawal latency to 10°C water was observed 3 days after oxaliplatin treatment (Fig. 1; $P < 0.05$ vs. vehicle).

Oxaliplatin-induced cold hypersensitivity in NHP over time

Robust cold hypersensitivity was observed after each i.v. infusion of 5 mg/kg oxaliplatin (Fig. 2). Beginning 1 day after oxaliplatin treatment, reduced withdrawal latencies to 10°C water were observed, but maximal reduction in latency was obtained 3 days after oxaliplatin treatment (Fig. 2A; $P < 0.05$ vs. baseline).

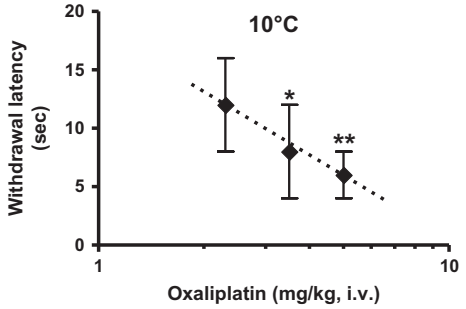

Figure 1. Dose–effect curve of oxaliplatin on tail withdrawal latencies in nonhuman primates. Tail withdrawal latencies to 10°C water were measured 3 days after a single i.v. infusion of oxaliplatin (2.3 mg/kg, 3.5 mg/kg, and 5.0 mg/kg). Values are expressed as the mean ± SEM; $n = 6$/dose. *$P < 0.05$, **$P < 0.01$ versus vehicle.

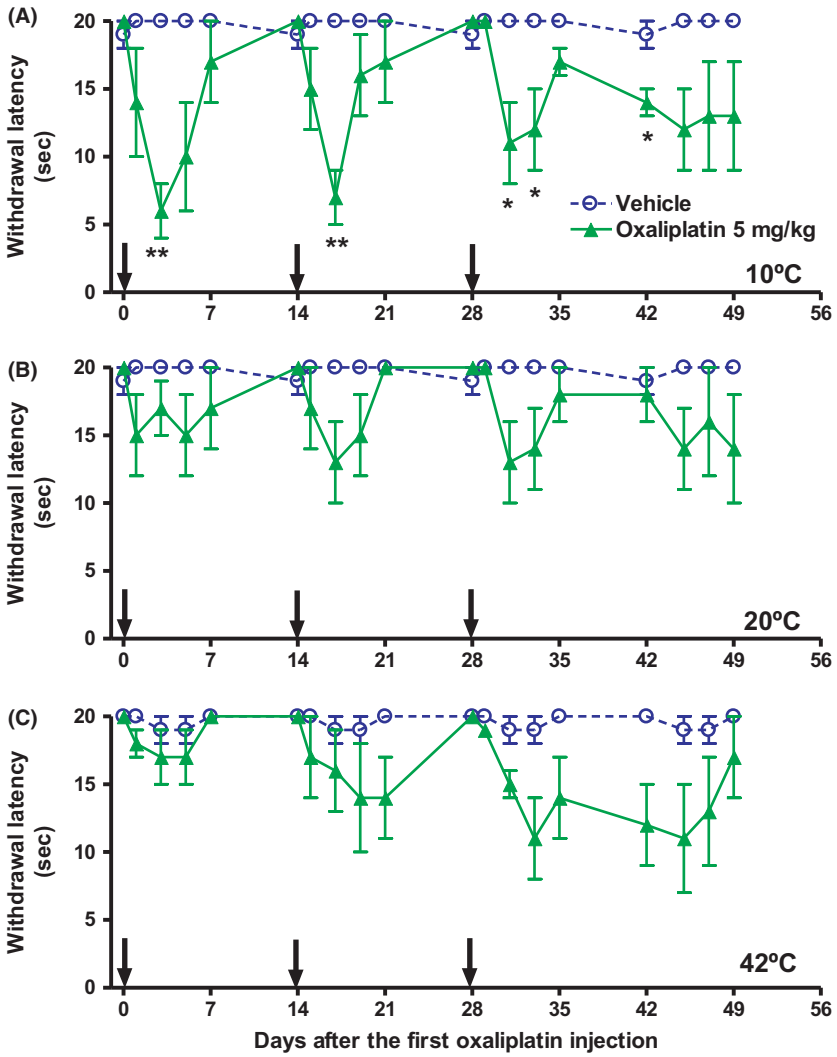

Figure 2. Effect of oxaliplatin treatment on tail withdrawal latency in nonhuman primates over time. Prior to oxaliplatin infusion, withdrawal latencies (in sec) to various temperatures of water were measured. The cut-off latency was 20 sec. Oxaliplatin (5 mg/kg) was intravenously infused three times (arrows), on days 0, 14, and 28. Withdrawal latencies were measured before each infusion and 1, 3, 5, and 7 days after each oxaliplatin infusion. The tail was immersed in (A) 10°C, (B) 20°C, and (C) 42°C water. Values are expressed as the mean ± SEM; n = 3/vehicle group; n = 5–6/oxaliplatin group. *$P < 0.05$, **$P < 0.01$ versus baseline.

Withdrawal latencies were reduced 5 days after oxaliplatin treatment, with a gradual recovery of withdrawal latencies to pretreatment baseline over time. Two weeks following oxaliplatin treatment, withdrawal thresholds were similar to that of vehicle-treated animals. A similar pattern of an acute, short-lasting cold hypersensitivity was observed following the second and third oxaliplatin treatments. All oxaliplatin-treated macaques developed some degree of cold hypersensitivity beginning with the first i.v. infusion – no oxaliplatin-treated macaque had a withdrawal latency of 20 sec. By contrast, macaques i.v. infused with vehicle did not exhibit a change in sensitivity to 10°C water.

Interestingly, there appeared to be a sustained cold hypersensitivity after the third oxaliplatin treatment (Fig. 2A). Withdrawal latencies were significantly reduced 3, 5, and 14 days after the third i.v. infusion (Fig. 2A; $P < 0.05$ vs. baseline). A trend of a sustained cold hypersensitivity 17 days after the third i.v. infusion was observed.

While withdrawal latencies to 20°C and 42°C water in oxaliplatin-treated macaques were slightly reduced, these decreases were not statistically significant (Fig. 2B, C; $P > 0.05$ vs. baseline). Vehicle-treated macaques did not show alterations in sensitivity to either 20°C or 42°C water.

Effects of clinical analgesics in NHP with oxaliplatin-induced cold hypersensitivity

Prior to drug administration, oxaliplatin-injected macaques showed decreased tail withdrawal latencies.

One hour following p.o. administration, duloxetine increased withdrawal latency (Fig. 3A). In fact, withdrawal latencies almost reached cut-off. A lower dose of duloxetine (10 mg/kg, p.o.) had no effect on tail withdrawal latency to 10°C water (data not shown).

By contrast, neither pregabalin nor tramadol had an effect on oxaliplatin-induced cold hypersensitivity (Fig. 3B, C); no effect of tramadol on cold hypersensitivity was observed at either 1 or 2 h after dosing.

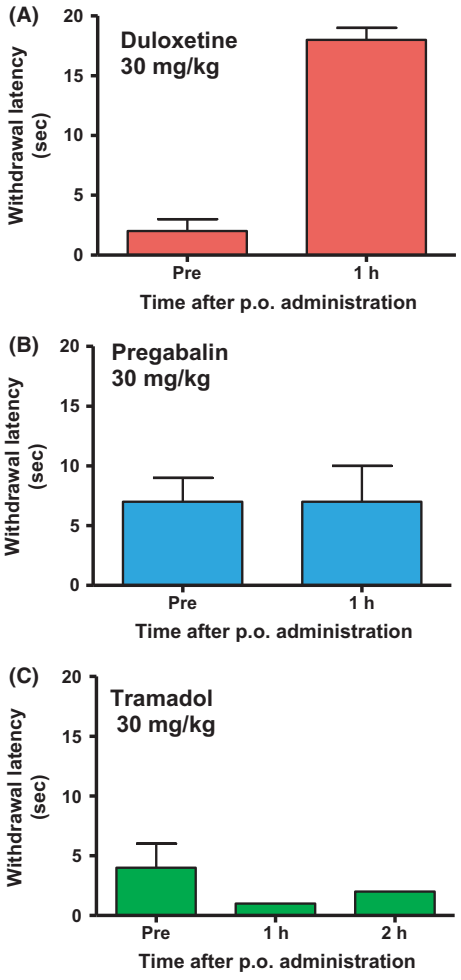

Figure 3. Effects of clinical analgesics on cold hypersensitivity in oxaliplatin-treated nonhuman primates. Baseline tail withdrawal latencies ("Pre") to 10°C water were measured 3 days and 17 days after oxaliplatin infusion (5 mg/kg, i.v.). Macaques were dosed with 30 mg/kg (p.o.) of either (A) duloxetine or (B) pregabalin and tested 1 h following dosing. (C) Macaques were dosed with 30 mg/kg (p.o.) tramadol and tested 1 and 2 h following dosing. Values are expressed as the mean ± SEM. n = 4/duloxetine; n = 4/pregabalin; n = 3/tramadol.

Health status of oxaliplatin-treated rats

While weight gain of oxaliplatin-treated rats over time was less than that of sham-treated rats ($P < 0.05$), the rats appeared to tolerate the currently used dose of oxaliplatin. None of the oxaliplatin-treated rats were excluded for health reasons and no mortalities were observed (see Fig. S1).

Oxaliplatin-induced cold hypersensitivity in rats over time

Prior to the first oxaliplatin treatment, rats showed little responsiveness to acetone applied to the hind paws. Three days after the first injection of oxaliplatin, about 4% of the rats injected with oxaliplatin demonstrated cold hypersensitivity, defined as a cold score ≥4 (Table 1). Because of the low number of rats that demonstrated cold hypersensitivity, analgesics were not tested in these rats. Rats injected with vehicle did not display increased responsiveness to acetone.

In the second group of oxaliplatin-treated rats, cold hypersensitivity emerged following six treatments of oxaliplatin, or 16 days after the first injection of oxaliplatin (Table 1). Rats that demonstrated cold scores ≥4 were used for drug testing – about 65% of all rats met this threshold. By contrast, sham-treated rats showed no response to acetone at any time point.

Effects of clinical analgesics in rats with oxaliplatin-induced cold hypersensitivity

Duloxetine, pregabalin, and tramadol significantly reduced cold scores in oxaliplatin-treated rats (Fig. 4; $P < 0.05$ vs. vehicle). By contrast, vehicle in oxaliplatin-treated rats did not significantly affect cold score.

Table 1. Short-term and long-term treatment with oxaliplatin in rats.

	Oxaliplatin treatments (5 mg/kg, i.p.)	Test day (days after the first oxaliplatin treatment)	Presence of cold hypersensitivity (%)
Short-term treatment	2	3	4
Long-term treatment	6	17	65

Presence of cold hypersensitivity indicated by a cold score ≥4.

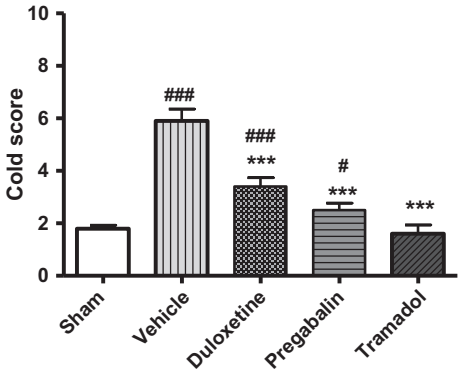

Figure 4. Effects of clinical analgesics on cold hypersensitivity in oxaliplatin-treated rats. Seventeen days after the first injection of oxaliplatin, oxaliplatin-injected rats were dosed with 30 mg/kg (p.o.) of either duloxetine, pregabalin, or tramadol and tested 1 h following dosing. $n = 10$/treatment group. Values are expressed as the mean ± SEM. $^{\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus sham; $^{***}P < 0.001$ versus vehicle.

While duloxetine and pregabalin suppressed pain-related behavior, the cold score in these animals was still higher than that of sham-treated rats (rats that received vehicle instead of oxaliplatin; $P < 0.05$ vs. sham). Tramadol was the only drug that fully suppressed cold hypersensitivity in oxaliplatin-treated rats. The pain-related behaviors in these rats were such that their response to acetone was similar to that of sham-treated rats ($P > 0.05$ vs. sham).

Discussion

Oxaliplatin-treated macaques demonstrated a robust acute or early onset cold hypersensitivity as indicated by significantly decreased withdrawal latency to 10°C water, which was reversed by duloxetine. By contrast, there was a lack of early onset cold hypersensitivity in rats following oxaliplatin treatment. Also, in cold hypersensitivity induced by repeated oxaliplatin treatment in rats, pregabalin and tramadol, as well as duloxetine, demonstrated significant antinociception. The overall differential pharmacology between NHP and rodents suggests species-specific mechanisms of oxaliplatin-induced neuropathy. If such is the case, then preclinical testing of novel treatments for CIPN and elaborating clinical CIPN mechanism will need to incorporate animals such as NHP.

In the nonhuman primate, the mean (±SD) peak plasma level of 5 mg/kg oxaliplatin (as inorganic platinum) after a 2 h i.v. infusion is 10.6 ± 2.6 μM (Jacobs et al. 2005). By contrast, since oxaliplatin has very limited ability to cross the blood–brain barrier, platinum CSF levels are about 2% of plasma levels (Jacobs et al. 2005). Thus, cold hypersensitivity is most likely a result of primary afferent neuron neurotoxicity (Jacobs et al. 2005; Park et al. 2011; Charest et al. 2013). Potential consequences of peripheral neurotoxicity include interference of ion channel function and disruption of primary afferent metabolism, which, upon repeated exposure to oxaliplatin, could lead to persistent axonal degeneration and eventually cell death (Jaggi and Singh 2012; Han and Smith 2013). A central component is possible in that oxaliplatin could accumulate in brain over repeated dosing – levels in brain following repeated dosing have yet to be reported.

In the current study, oxaliplatin induced a robust cold hypersensitivity in both macaques and rats, but the onset of symptoms greatly differed. All macaques exhibited a robust, dose-dependent, early onset cold hypersensitivity, paralleling clinical findings (Miltenburg and Boogerd 2014; Zedan et al. 2014). A possible prolonged cold hypersensitivity was observed after the third infusion of oxaliplatin, suggestive of a chronic neuropathic pain that persists long after the last dose of oxaliplatin. It is likely that a persistent cold hypersensitivity would appear after further infusions (Carozzi et al. 2015).

By contrast, few of the rats in the current study demonstrated an early-onset cold hypersensitivity following short-term treatment with oxaliplatin (two injections), despite use of a previously described treatment schedule (Sakurai et al. 2009). A number of studies have shown an emergence of early-onset cold hypersensitivity following a single treatment with oxaliplatin, but the true extent to which oxaliplatin induces neuropathy in rodents is not entirely known (Ling et al. 2008; Aoki et al. 2014; Balayssac et al. 2014; Zhao et al. 2014). Age, oxaliplatin dose, and behavioral test could be key considerations (further elaboration of these factors is explained in Table S1). The current findings also suggest that the acute, and possibly chronic, sensory changes induced by oxaliplatin could differ from species to species.

A late or chronic cold hypersensitivity was observed in rats following multiple oxaliplatin treatments (six injections) which was sensitive to duloxetine, pregabalin, and tramadol. Cold hypersensitivity in the rat, evoked with either acetone or a cold stimulus, appears to be sensitive to a range of pharmacotherapeutics (Ling et al. 2008; Authier et al. 2009; Aoki et al. 2014; Balayssac et al. 2014). Small-diameter primary afferents, C-fiber and A-delta, are believed to mediate abnormal temperature sensitivity following nerve injury or neurotoxicity in rats (Colburn et al. 2007; Leith et al. 2010; Xiao et al. 2012). Abnormal activity expressed by these afferents is suppressed by pregabalin, duloxetine, and tramadol (Haeseler et al. 2006; Wang et al. 2010; Chen et al. 2013). The rodent findings suggest that inhibiting abnormal

small-diameter primary afferent activity could be useful in the treatment of oxaliplatin-induced neuropathic pain.

In contrast to the rat findings, oxaliplatin-induced neuropathic pain in the macaque was not sensitive to either pregabalin or tramadol. The lack of an acute analgesic effect of pregabalin and tramadol in the oxaliplatin-treated macaque could be due to pharmacokinetics. However, significant analgesia has been observed within 2 h of a single dose (150 mg) of pregabalin in patients with painful herpes zoster (Jensen-Dahm et al. 2011). This dose is equivalent to 2.5 mg/kg or 7.8 mg/kg in macaques (Food and Drug Administration 2005). The time to maximal tramadol concentration in serum (T_{max}) in rhesus macaques is 120 min (Kelly et al. 2015). The maximum daily clinical dose of tramadol is 6.7 mg/kg or 20.7 mg/kg in the macaques (Food and Drug Administration 2005). Thus, extrapolating from the clinical data, the dose and the timing of behavioral assessment of the current study were sufficient such that efficacy could have been observed. It is possible that pregabalin and tramadol could be efficacious in the macaque oxaliplatin-induced neuropathy model following extended treatment.

Alternatively, it is possible that the mechanism underlying oxaliplatin-induced cold hypersensitivity in macaques is distinct from that of rats. While rodents have a number of advantages as a preclinical model species, they do have a number of limitations as well, including their phylogenetic distance from humans (Kumar and Hedges 1998). One consequence of this is that pain-related targets in one species have strikingly different expression patterns or functions from that of the human homolog (Upadhyay et al. 2011; Chen et al. 2013; Hirai and Hama 2014). The species difference in molecular biology could be one reason for the disconnection between significant efficacy observed in rodent models of oxaliplatin-induced neuropathic pain and the failure to replicate efficacy in patients.

The only drug that has demonstrated significant efficacy against oxaliplatin-induced neuropathic pain in a phase III clinical trial so far is duloxetine (Smith et al. 2013; Hershman et al. 2014). In the current study, a single dose of duloxetine acutely alleviated cold hypersensitivity in both macaques and rats. Whether duloxetine has an acute analgesic effect in oxaliplatin-treated patients has not been reported. In diabetic neuropathic pain, meaningful clinical analgesia has been reported within 1 day of taking a single dose of duloxetine (60 mg) (Pritchett et al. 2007). It is entirely unknown whether the mechanism of action is primarily due to noradrenergic and serotonergic reuptake inhibition in the CNS or block of peripherally expressed sodium channels or a combination of both (Haeseler et al. 2006). The channel blocking activity of duloxetine, however, could explain the rapid onset of efficacy observed in neuropathic pain patients. The current finding raises the possibility of elaborating the mechanism of duloxetine's analgesic effect on oxaliplatin-induced neuropathic pain a nonrodent species. A combination of invasive procedures, such as electrophysiology and in vivo microdialysis, and noninvasive in vivo imaging could provide useful information regarding duloxetine's site of action and mechanism and set the stage for the development of novel analgesics (Upadhyay et al. 2011).

Limitations

While the current data suggest species-related differences in response to oxaliplatin treatment, one should consider methodological differences, namely, i.v. versus i.p. oxaliplatin dosing, tail immersion–cool water versus hind paw acetone and female versus male animals, between the macaque and rat, respectively, experiments. Interestingly, all rat studies that evaluated analgesics on early onset cold hypersensitivity utilized i.p. oxaliplatin dosing. It is curious that i.p. dosing is utilized in rats rather than i.v. dosing, the clinical route of administration. Nonetheless, all studies report robust early onset hypersensitivity following i.p. oxaliplatin dosing and clinically relevant levels of oxaliplatin (as inorganic platinum) in plasma (Zanardelli et al. 2014).

Related to dosing is the differing duration of exposure to oxaliplatin between macaques and rats, in that analgesics were assessed in rats after they had received six doses of oxaliplatin. Perhaps a lack of efficacy of pregabalin and tramadol as observed in the macaques could have been observed in early onset cold hypersensitivity in the rats. However, significant antinociceptive effects of gabapentin, pregabalin, and tramadol have been observed on cold hypersensitivity induced by a single injection of oxaliplatin in rodents (Ling et al. 2008; Aoki et al. 2014; Deuis et al. 2014; Zhao et al. 2014). These previous findings, combined with the current findings in the macaque, support our contention that there is a potential difference in the efficacy of therapeutics based on species.

In the case of cool water (tail) versus acetone (paw) stimulation, it possible that the response evoked by either stimuli is mediated by a common pain mechanism. This is inferred by the finding that pregabalin ameliorates cold hypersensitivity, whether the stimulus is a cold (8°C) metal probe applied to the hind paw (Aoki et al. 2014) or 10°C water tail immersion (Ling et al. 2008). Combined with the current finding that pregabalin ameliorates acetone-evoked hind paw cold hypersensitivity, pregabalin appears to modulate the mechanism mediating oxaliplatin-induced cold hypersensitivity – whether it is evoked by acetone (cooling?) or cold stimulation. Fur-

thermore, in peripheral nerve-injured rats, efficacy of gabapentin was observed whether mechanical allodynia of the tail (Back et al. 2004) or hind paw (Hunter et al. 1997) was assessed, which suggests that the "neuropathic mechanism" that mediates allodynia in the tail and hind paw is similar. Further testing of other sensory modalities and stimuli is needed to either support or refute this contention (Attal et al. 2009).

There is growing impetus to assess pain in female nonhuman animals as gender differences to pain and analgesics have been documented (Fillingim and Ness 2000). With respect to oxaliplatin-induced neuropathic pain, gender may not be a significant risk factor (Attal et al. 2009) and there are no differences between gender in terms of oxaliplatin pharmacokinetics (Graham et al. 2000). Certainly, however, further studies are warranted, including elaboration of a possible effect of gender on analgesics such as duloxetine in oxaliplatin-induced neuropathic pain (Smith et al. 2013).

Conclusion

Both laboratory scientists and clinicians face a number of barriers in turning promising laboratory findings into useful clinical treatments (Lowenstein and Castro 2009). From the preclinical side, the current nonhuman primate model of CIPN could be utilized with the aim of overcoming the translational barrier. Perhaps the macaque could be used to confirm efficacy of the most promising treatments. Under such a scheme, the work of developing treatments for patients will become more complex with the inclusion of a complex animal species, but ultimately, it is expected that patients who need effective treatments will benefit.

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Disclosures

All authors are employees of Hamamatsu Pharma Research, Inc.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effect of oxaliplatin treatment on body weight in rats over time.
Table S1. Early onset cold allodynia in rats: weight, oxaliplatin dose, and behavioral test.