Neuropharmacology 79 (2014) 432–443

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Differential pharmacological alleviation of oxaliplatin-induced hyperalgesia/allodynia at cephalic versus extra-cephalic level in rodents

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ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form
6 December 2013
Accepted 9 December 2013

Keywords:
Oxaliplatin
Neuropathic pain
Mechanical allodynia
Cold hyperalgesia
Inflammatory pain
Rodents

ABSTRACT

Previous data showed that neuropathic pain induced by mechanical lesion of peripheral nerves responds differently to alleviating drugs at cephalic versus extracephalic level. Because neuropathic pain evoked by anti-cancer drugs differs from that triggered by mechanical nerve lesion, we investigated whether differences between cephalic and extracephalic levels could also be characterized in rodents rendered neuropathic by treatment with the anti-cancer platinum derivative oxaliplatin.

C57BL/6J mice received two injections and Sprague-Dawley rats three injections of oxaliplatin (10 mg/kg, i.p.) or its vehicle, with three days intervals. Supersensitivity to mechanical (von Frey filaments), cold (acetone drop) and chemical/inflammatory (formalin) stimulations was assessed in vibrissae and hindpaw territories. Transcripts of neuroinflammatory markers were quantified by real-time RT-qPCR in rat ganglia and central tissues.

Oxaliplatin induced mechanical allodynia, cold hyperalgesia and chemical/inflammatory supersensitivity at both hindpaw and vibrissal levels in mice and rats. Acute treatment with gabapentin (30 mg/kg i.p.), morphine (3 mg/kg s.c.) or the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.16 mg/kg s.c.) significantly reduced oxaliplatin-induced supersensitivity in hindpaw but not vibrissal territory. In contrast, the antimigraine drugs naratriptan (0.1 mg/kg s.c.) and olcegepant (0.6 mg/kg i.v.) decreased oxaliplatin-induced supersensitivity in vibrissal territory only. Among the various markers investigated, only TRPA1 transcript was upregulated in ganglia of oxaliplatin-treated rats.

These data showed that oxaliplatin induced supersensitivity to various stimuli in both cephalic and extra-cephalic territories in rodents. Regional differences in the efficacy of drugs to alleviate oxaliplatin-induced allodynia/hyperalgesia further support the idea that mechanisms underlying neuropathic pain have peculiarities at cephalic versus extra-cephalic level.

1. Introduction

Neuropathic pain is a major public health problem because available treatments produce incomplete relief, and have dose-limiting side effects (Baron et al., 2010). Furthermore, anti-neuropathic pain drugs can have different efficacy to alleviate symptoms at cephalic versus extra-cephalic levels (Attal et al., 2010; Robinson et al., 2004) in line with clear-cut data showing that chronic injury-induced orofacial pain has specific characteristics compared with extra-cephalic nerve injury-induced pain mediated through the spinal nociceptive system (Fried et al., 2001; Hargreaves, 2011). Previous studies in rats with chronic constriction injury (CCI) to either the sciatic nerve (SN) or the infraorbital nerve (ION) confirmed that pharmacological and pathophysiological differences exist between extra-cephalic and cephalic pain models. Thus, low doses of morphine, gabapentin and tetrodotoxin significantly reduced hindpaw allodynia in CCI–SN rats but were essentially ineffective against allodynia in vibrissal territory of CCI–ION rats, clearly indicating that these drugs are more effective at extra-cephalic compared to cephalic level (Christensen et al., 2001; Kayser and Christensen, 2000; Kayser et al., 2010). In contrast, antimigraine drugs such as triptan agonists at 5-$HT_{1B/1D}$ receptors

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http://dx.doi.org/10.1016/j.neuropharm.2013.12.011

and CGRP receptor antagonists such as olcegepant alleviated allodynia in CCI–ION rats, but were ineffective in CCI–SN rats (Kayser et al., 2002, 2011; Michot et al., 2012), in line with other studies showing that these drugs inhibit trigeminal pain but exert no pain alleviating effects, even at high doses, in various rodent models of extracephalic pain (Connor et al., 1997; Goadsby and Knight, 1997; Ottani et al., 2004; Skingle et al., 1990). In addition to these pharmacological differences, investigations aimed at identifying physiopathological mechanisms demonstrated that nerve ligation-induced microglia activation and cytokines' upregulation in both sensory ganglia and central tissues exhibit different characteristics in CCI–ION vs CCI–SN rats (Latrémolière et al., 2008).

Anticancer drugs are cytotoxic agents known to cause nerve injury at the origin of neuropathic pain (Balayssac et al., 2011; Windebank and Grisold, 2008). Among these drugs, oxaliplatin is the preferred agent to reduce metastasic colorectal cancer, notably because its effectiveness is not opposed by excessive nephrotoxicity (as is the case with cisplatin) or hematotoxicity (as with carboplatin). However, neurotoxicity frequently occurs in patients treated with oxaliplatin, and is a dose-limiting side effect of this drug (Extra et al., 1998; Wolf et al., 2008). During anticancer treatment, oxaliplatin strongly accumulates in dorsal root ganglia and induces two types of neurological symptoms. Acute symptoms consisting of dysesthesia and paresthesia exacerbated by cold stimulation appear just after starting the treatment and persist for a few days (Park et al., 2008, 2009; Pasetto et al., 2006). Then, under chronic treatment conditions, sensory loss and pain occur, and these symptoms of chronic neuropathy can persist for several months after cessation of oxaliplatin administration (Cavaletti et al., 2001; Extra et al., 1998). Furthermore, consistent reports emphasized that oxaliplatin-induced pain is located not only in limbs' extremities (extra-cephalic territory) but also in cephalic area (jaw, eyes and perioral area) (Leonard et al., 2005).

These clinical observations led us to investigate whether repeated administration of oxaliplatin would lead to neuropathic pain at both extra-cephalic and cephalic levels thereby offering the opportunity to assess whether drugs which were reported to exert different pain alleviating effects at these two levels, but in distinct animal models (Connor et al., 1997; Goadsby and Knight, 1997; Kayser et al., 2002, 2010, 2011; Skingle et al., 1990), would act similarly in the *same* animal. We thus tested the respective efficacy of morphine, gabapentin, 8-hydroxy-2-(N,N-di-propyl-amino-tetralin) (8-OH-DPAT), naratriptan and olcegepant, at effective doses in validated pain tests (Bardin et al., 2001; Christensen et al., 2001; Idanpaan-Heikkila and Guilbaud, 1999; Kayser et al., 2002; Michot et al., 2012; Omori et al., 2009), on hyperalgesia and/or allodynia at both hindpaw and vibrissal territory levels in oxaliplatin-pretreated rats and mice. RT-qPCR measurements of transcripts encoding neuroinflammatory markers were also performed to further characterize physiopathological mechanisms underlying oxaliplatin-induced neuropathic-like pain symptoms at cephalic vs extra-cephalic levels in rats.

2. Materials and methods

2.1. Animals

All experiments were performed in full conformity with the Ethical Guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983), and strictly followed institutional guidelines in compliance with French (Service de Protection et Santé Animales, Préfecture de Police, Authorization nb B-75-116 to M.H.) and European Communities (86/609/EEC, 24 November 1986) Directives for use of animals in neuroscience research. Furthermore, in order to minimize animal suffering, only the most informative nociceptive tests were performed when our objective was to confirm that rats became allodynic and/or hyperalgesic after oxaliplatin treatment. In particular, the formalin test was not performed in oxaliplatin-treated rats that had already been assessed for allodynia/hyperalgesia using mechanical and thermal tests (see Results 3.3).

Adult male C57BL/6J mice (8 weeks-old on arrival) and Sprague-Dawley rats (150–175 g body weight on arrival) were purchased from Charles River (Lyon, France). Animals were allowed to habituate to the housing facilities (ambient temperature: 22 ± 1 °C; relative humidity: 60%; food and water *ad libitum*; 12 h light/dark cycle, with lights on at 7:00 A.M.) for at least one week before experiments.

2.2. Pharmacological treatments

Oxaliplatin solution (Eloxatin®) was diluted with 0.9% NaCl down to 1.0 mg/ml for i.p. injections to mice (1 ml/100 g body weight), and to 2.5 mg/ml for i.p. injections to rats (0.4 ml/100 g body weight). Oxaliplatin at 10 mg/kg or saline was administered twice in mice, on day 0 and day 3, and three times in rats, at days 0, 4 and 7, at 10:00 A.M. This dosage of oxaliplatin was in the range of those used in previous studies also aimed at inducing neuropathy in rodents (Cavaletti et al., 2001; Ling et al., 2007; Ta et al., 2009).

Acute treatments with morphine (3 mg/kg s.c.), gabapentin (30 mg/kg i.p.), 8-hydroxy-N,N-dipropylamino-tetralin (8-OH-DPAT, 0.16 mg/kg s.c.), naratriptan (0.1 mg/kg s.c.), olcegepant (0.6 mg/kg i.v.) or vehicle were performed 8 days after the first oxaliplatin injection in mice, whereas this time interval was of 15 days in rats. The doses and routes of administration chosen for these drugs were previously shown to be effective at their respective molecular targets (Bardin et al., 2001; Christensen et al., 2001; Idanpaan-Heikkila and Guilbaud, 1999; Kayser et al., 2002; Michot et al., 2012; Omori et al., 2009).

2.3. Von Frey filament test

2.3.1. In rats

Rats that had been repeatedly treated twice with oxaliplatin or saline under the conditions described above, then acutely with a given drug (see 2.2.) or its vehicle, were subjected to mechanical stimulation with von Frey filaments to assess allodynia both at hindpaw (extra-cephalic territory) and vibrissal pad (cephalic territory) level (Latrémolière et al., 2008). Each rat was habituated for 1–2 h on a metal mesh floor, under a small plastic cage (35 × 20 × 15 cm). Tactile allodynia was determined with a graded series of von Frey filaments (Bioseb, Chaville, France) producing a bending force ranging from 0.4 to 10.0 g for vibrissal pad and from 6 to 60 g for hindpaw. The stimuli were applied in each territory beginning alternatively in vibrissal pad or in hindpaw with a 10 min interval between the tests. Each filament was applied three times (3 s apart) always starting with the filament producing the lowest force. For vibrissal pad, aversive nociceptive behavior consisted of either a brisk withdrawal of the head, an attack or an escape reaction. For hindpaw, relevant behavior consisted of either a brisk paw withdrawal and/or an escape attempt. The minimal pressure causing at least one of these responses allowed determination of the mechanical response threshold. The filaments of 10 g for vibrissal pad and 60 g for hindpaw were chosen as the cut-off thresholds to prevent tissue-injury. In the pre-oxaliplatin treatment tests, stimulation with the 10 g or 60 g filament (of vibrissal pad or hindpaw, respectively) did not induce any nociceptive behavior in the majority of the rats (>90%). In order to avoid nonspecific responses, only these rats were included in the study.

2.3.2. In mice

Excessive head agitation in mice did not allow reliable determinations of mechanical response threshold using von Frey filaments at cephalic level, and the test could be carried out at hindpaw level only. Each mouse was placed under a transparent plexiglas box (7 × 12.5 × 17 cm) on a metal mesh floor and allowed to acclimate for 30 min before testing. Calibrated von Frey filaments (bending force ranging from 0.4 to 6.0 g, Bioseb) were applied in ascending order, using a single, steady, 1–2 s application to the plantar surface of the hindpaw. The minimal pressure causing hindpaw withdrawal allowed the determination of the mechanical response threshold (Kayser et al., 2007). In each mouse, threshold value was determined three times with an interval of at least 2 min.

2.4. Acetone drop test

The acetone drop test (Kim et al., 1997; Smith et al., 2004) was used to assess cold hyperalgesia-like responses in hindpaw and/or vibrissae territories in both oxaliplatin- versus saline-treated rats and mice after acute administration of various drugs or their vehicles.

2.4.1. In rats

Rats were habituated for 1 h on a metal mesh floor, under small plastic cages (35 × 20 × 15 cm). An acetone drop was formed at the tip of a 1 ml syringe and then gently applied onto the plantar surface of a hindpaw. Acetone-induced paw flinches were counted for 2 min. Each test consisted of applying acetone 5 times (with a 5 min interval between successive applications), and the cumulated response (total number of hindpaw flinches for the 5 successive applications) was calculated.

2.4.2. In mice

Mice were placed either on a metal mesh floor, under a transparent plexiglas box (7 × 12.5 × 17 cm) for hindpaw stimulation, or in a plexiglas box (11.5 × 7.5 × 7 cm) for vibrissal pad stimulation, and allowed to acclimate for 30 min. An acetone drop