Acute cold allodynia induced by oxaliplatin is attenuated by amitriptyline

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INTRODUCTION

The Neuropathic Pain Special Interest Group (NeuPSiG) of the International Association for the Study of Pain (IASP) defines neuropathic pain as pain caused by a lesion or disease that affects somatosensory nervous system (Parisi et al. 2017). This type of pain is regarded as a chronic disease that has a substantially negative effect on patients’ quality of life and is associated with high socioeconomic burden (Finnerup et al. 2015).

Despite a large variety of causes that comprise metabolic, traumatic or iatrogenic factors, including those related to adverse drug reactions, neuropathic pain is now considered to be a distinct clinical entity (Jensen and Finnerup 2014, Manji 2013). Available meta-analyses reporting the incidence of adverse drug reactions in patients revealed that drug-induced neuropathies are rare and unlikely to be fatal, with the incidence between 2 and 4% (Manji 2013, Vilholm et al. 2014), most often being manifested as mild sensory paresthesias not requiring specific treatment. However, it should be noted that if drug-related neuropathies are associated with pain, specific treatment with analgesic drugs is usually required (Manji 2013).

The number of drugs for which drug-induced neuropathic pain episodes have been reported is still increasing (Vilholm et al. 2014). Among these drugs are anti-tumor drugs: platinum-based drugs, vincristine, paclitaxel, thalidomide and bortezomib which are all frequently reported to induce severe pain and other intolerable symptoms of chemotherapy-induced periph-
eral neuropathy (CIPN). Of note, although numerous analgesic drugs are currently used to treat neuropathic pain of various origins, including CIPN-related pain, it is estimated that about 30-40% of patients are pharmaco-resistant to available treatment options (Finnerup et al. 2005, Gagnon et al. 2003) and the treatment of CIPN remains largely ineffective (Torrance et al. 2013). As a consequence, this may lead to chemotherapeutic drug dose reduction, or even treatment discontinuation (Han and Smith 2013).

Oxaliplatin is a third-generation platinum-based anti-tumor drug used to treat advanced colorectal cancer. Compared to other platinum-based drugs, it has a better safety profile featuring a lower incidence of hematological adverse effects and reduced gastrointestinal toxicity. However, it has been demonstrated that in approximately 95% of patients oxaliplatin causes CIPN and severe neuropathic pain episodes which are due to toxic effects of this drug and/or its metabolite—oxalate, on the somatosensory system and nerve hyperexcitability (Ewertz et al. 2015, Manji 2013, Sakurai et al. 2009). Hence, apart from its broad application in human and veterinary oncology, oxaliplatin is also used under experimental conditions to induce a rodent model of CIPN that could be utilized in the search for novel analgesics for neuropathic pain.

Oxaliplatin-induced CIPN-related neuropathic pain is characterized by a lowered mechanical and thermal nociceptive threshold, i.e., tactile allodynia and thermal (cold) allodynia, respectively. To assess the effect of oxaliplatin on tactile allodynia von Frey filaments are used in laboratories worldwide. In contrast to this, the plethora of methodological differences in protocols used for the assessment of oxaliplatin-induced cold allodynia in rodents is striking. Researchers use distinct temperatures ranging from 0°C (Pevida et al. 2013), through 2°C (Mika et al. 2007, Nakanishi et al. 2016), 4°C (Berrocoso et al. 2011, Hache et al. 2015, Masocha and Parvathy 2016) to 5°C (Sambasevam et al. 2017, Zhao et al. 2012), different time-points at which they assess animals’ nociceptive threshold—from 2 h (Zhao et al. 2012) to 7 days after oxaliplatin injection (Hache et al. 2015, Zhao et al. 2012), and distinct behavioral measures are taken as end-points, i.e., paw withdrawal latencies (e.g., paw licking, paw shaking) (Pevida et al. 2013), jumping behavior (Hache et al. 2015), or paw lifting (Mika et al. 2007), and escape behaviors are graded with a score from no response to vigorous activity (i.e., jumping) (Zhao et al. 2012) or by the duration of the nocifensive response (Nakanishi et al. 2016).

In the light of these methodological differences, it is difficult to analyze and compare the analgesic efficacy of drugs used for the attenuation of oxaliplatin-induced neuropathic pain. Hence, in the present study we investigated the influence of various temperatures used on the ability of single-dose oxaliplatin to induce cold allodynia in CD-1 mice, and we also assessed the effectiveness of amitriptyline to reduce oxaliplatin-induced cold allodynia in mice. As mentioned above, recent studies have indicated that neuropathic pain caused by oxaliplatin might be related to the accumulation of its toxic metabolite (oxalate), which binds to voltage-gated sodium channels (Na+), and may be particularly responsible for cold allodynia effects (Ewertz et al. 2015, Sakurai et al. 2009). Amitriptyline is a tricyclic antidepressant drug with well-documented Na+-inhibitory properties (Horishita et al. 2017). Thus, we investigated the use of amitriptyline in CIPN-related pain caused by oxaliplatin might be a potential treatment option for this condition. Previous studies have shown that amitriptyline reduced tactile allodynia in several other neuropathic pain types (Kremer et al. 2016, Sanna et al. 2017, Sawynok and Zinger 2016), but there are conflicting data regarding its effect on cold allodynia, particularly in patients treated with platinum derivatives (Ewertz et al. 2015, Sanna et al. 2012).

METHODS

Animals and housing conditions

Behavioral experiments were carried out at the Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College in Krakow, Poland. Adult male albino Swiss (CD-1) mice weighing between 18 g and 22 g were used in the experiments. Prior to experiments the animals were kept in groups of 10 mice in home cages at room temperature (22±2°C), under a (12:12) light/dark cycle. Before the assays the mice had free access to food and water. The ambient temperature of the experimental room and the humidity (55±5%) were kept consistent throughout the tests. For behavioral experiments the animals were selected randomly. Each group consisted of 6-10 animals. The experiments were performed between 8 AM and 2 PM. Immediately after the assay, the animals were euthanized by cervical dislocation. All procedures were approved by the Local Ethics Committee of the Jagiellonian University in Krakow (4/2016) and the treatment of animals was in full accordance with ethical standards laid down in respective Polish and EU regulations (Directive No. 86/609/EEC).

Chemicals

To induce CIPN, oxaliplatin (Tocris Bioscience, Germany) prepared in 5% glucose solution (Polfa Kutno, Poland)
was administered intraperitoneally as a single dose of 10 mg/kg. Amitriptyline was purchased from Sigma Aldrich (Poland). In order to assess its effect on cold allodynia in neuropathic mice treated with oxaliplatin, amitriptyline was dissolved in 0.9% natrium chloride solution (Polfa Kutno, Poland) and injected intraperitoneally at doses of 1, 2.5 and 10 mg/kg.

Behavioral testing

Effect of oxaliplatin on cold allodynia (cold plate test)

The ability of oxaliplatin to induce cold allodynia was assessed in the cold plate test. This test was performed using the cold plate apparatus (hot/cold plate, Bioseb, France) set at three distinct temperatures of the cold plate: 1°C, 2.5°C and 4°C. To assess how oxaliplatin affects the cold nociceptive threshold of mice exposed to these various temperatures, the mice were divided into separate experimental groups that were tested 2 h, 3 h, 4 h and 6 h after oxaliplatin injection (assessment of acute-phase cold allodynia). Each of these groups was subjected to the cold plate assay again 24 h, 72 h and finally 7 days later to establish the impact of oxaliplatin on late-phase cold allodynia (Fig. 1A).

Effect of amitriptyline on cold allodynia in oxaliplatin-treated mice

Antiallodynic properties of amitriptyline in the early-phase of cold allodynia (i.e., on the day of oxaliplatin administration) and in the late phase of oxaliplatin-induced cold allodynia (i.e., 7 days after oxaliplatin injection; Fig. 1B) were assessed at one temperature set-point chosen based on the results obtained in the previous experiment.

In order to measure the effects of oxaliplatin and amitriptyline in the cold plate test, the animals were first tested to obtain baseline latencies to pain reaction before oxaliplatin injection (referred to as '0 h', 'before ox-
aliplatin latencies'). Then, oxaliplatin was injected and latencies to pain reaction (i.e., lifting, biting, shaking of hind paws, jumping, and movement deficits) were measured again at specific time-points (‘pre-drug latencies’). Next, amitriptyline was administered and 60 min later post-drug latencies to pain reaction were collected. In this assay, a cut-off time of 60 s was established to avoid potential paw tissue damage and animals not responding within 60 s were removed from the apparatus and assigned a score of 60 s.

Data analysis

Analysis of the results was performed using GraphPad Prism software (v.5.0, CA, USA). Numerical results are expressed as the mean ± SEM. Statistical analysis was carried out by using one-way analysis of variance (ANOVA), followed by Dunnett’s or Tukey’s post-hoc comparisons. P<0.05 was considered significant.

RESULTS

Effect of oxaliplatin on the development of cold allodynia

In the cold plate test with the temperature set at 1°C oxaliplatin showed an overall effect (F_{7,85}=15.50, P<0.0001). As shown in Fig. 2A, 3 h after oxaliplatin administration a significant (P<0.001) decrease in latency time to pain reaction was noted and this effect was maintained until the end of the testing period.

A temperature of 2.5°C also revealed an overall effect of oxaliplatin on cold allodynia in mice (F_{7,77}=21.19, P<0.0001). A significant (P<0.001) reduction in latency time to pain reaction was observed 2 h after oxaliplatin injection and this effect was maintained until the end of the testing period (Fig. 2B).

A temperature of 4°C also revealed oxaliplatin’s ability to induce cold allodynia in mice (F_{7,80}=21.23, P<0.0001). A significant (P<0.001) reduction of latency time to pain reaction was observed 3 h after oxaliplatin injection and this effect was maintained until the end of the testing period on day 7 (Fig. 2C).

Antiallodynic activity of amitriptyline in oxaliplatin-treated mice

In the next stage of the study we assessed the effect of amitriptyline at three different doses (1 mg/kg, 2.5 mg/kg and 10 mg/kg) on cold nociceptive threshold in oxaliplatin-treated mice. The previous results obtained for oxaliplatin showed that onset of symptoms of cold allodynia appeared earliest in mice exposed to 2.5°C, thus we used this temperature for further tests that aimed to establish antiallodynic properties of amitriptyline. We assessed the effect of amitriptyline during early-phase cold allodynia 1 h after amitriptyline administration (i.e., 4 h after oxaliplatin injection). The effect of this drug on late-phase cold allodynia was established 1 h after amitriptyline administration on day 7 after oxaliplatin injection.

Analysis of variance showed a significant overall effect of treatment (F_{14,135}=7.676, P<0.0001). Post hoc analysis demonstrated that oxaliplatin lowered the cold nociceptive threshold and caused significant (P<0.05) early-phase and late-phase cold allodynia in all groups tested and amitriptyline reduced cold allodynia in ox-
Oxaliplatin-treated mice. It is noteworthy that this antial- lodynic activity of amitriptyline was significant only for doses of 2.5 and 10 mg/kg (P<0.01 and P<0.05, respectively), and this effect was observed in the early phase (4 h after oxaliplatin injection, i.e., 1 h after amitriptyline injection), but not in the late phase (7 days after oxaliplatin administration) (Fig. 3).

**DISCUSSION**

The results of the present study demonstrated that oxaliplatin lowered the pain sensitivity threshold for cold nociception in mice and this effect was observed at temperatures ranging from 1°C to 4°C. We also showed that amitriptyline, likely due to its Na+-blocking properties, was able to attenuate early-phase cold allodynia caused by this platinum derivative but it had no effect on cold allodynia in the late phase of neuropathy.

Pain threshold is a highly subjective characteristic for each individual, thus for its assessment, as well as for the establishment of efficacy of analgesic drugs, reliable methods are essential. The use of oxaliplatin as a tool to induce neuropathic pain and to investigate the efficacy of analgesics is important because this anti-tumor drug is widely used for the treatment of various types of carcinoma (Petrioli et al. 2008). In line with this, the prevalence of oxaliplatin-induced adverse effects, including neuropathic pain, is high (Petrioli et al. 2008). In contrast to types of neuropathic pain other than those related to CIPN, e.g., diabetic neuropathic pain, the guidelines and algorithms for the management of oxaliplatin-induced neuropathic pain are not clear and there are only a few drugs, e.g., duloxetine, (Smith et al. 2013) that have well-established antiallodynic efficacy in oxaliplatin-treated patients. This necessitates the search for novel treatment options to alleviate oxaliplatin-induced neuropathic pain.

The use of oxaliplatin in experimental pharmacology has several advantages over other anti-tumor drugs used to model neuropathic pain in animals. Firstly, unlike other anti-tumor drugs such as vincristine, which requires repeated administrations to lower pain threshold (Gong et al. 2016), the use of oxaliplatin for pain studies is much more convenient as this drug is able to induce neuropathic pain after only a single-dose administration. Secondly, in contrast to paclitaxel which requires the use of solubilizers (Cremophor EL, absolute ethanol) for its preparation (Parvathy and Masocha 2015, Salat and Filipek 2015), oxaliplatin solutions can easily be prepared by dissolving in a glucose solution. Thirdly, oxaliplatin also produces acute neuropathy due to an effect of the oxalate salt on axonal sodium channels (Sakurai et al. 2009) and this effect is not seen with other platinum-complex agents or with any other chemotherapeutic drugs (Xiao et al. 2012). Fourthly, as with many other drugs inducing neuropathic pain, oxaliplatin induces tactile allodynia, mechnano-hyperalgesia, but it also causes cold hypersensitivity (cold allodynia), which is rather unique among neuropathic pain-inducing drugs.

Since there is no effect of oxaliplatin on heat sensitivity (Xiao et al. 2012), in our study we used the cold plate test as a simple method to assess the development of thermal (cold) allodynia in mice. In the first stage of the present experiment we wanted to determine if all of the temperatures most frequently used at the pre-clinical stage of drug development (for mice: 1°C - 4°C, for details please see the introduction section) could be used to assess cold allodynia after oxaliplatin injection. Since thermal methods of evaluation can be significantly biased by learning in mice subjected to the cold or hot plate tests (Czopek et al. 2016), we used separate groups of mice for each temperature tested to limit this undesirable effect. We found that the development of cold allodynia could be observed as early as 2 h after oxaliplatin administration. It should be noted that at this time-point the effect was statistically significant.
only in mice tested at 2.5°C. For all temperatures tested, early-phase cold allodynia was fully developed 3 h after oxaliplatin injection, thus this time-point was chosen for further experiments that involved measurements of pre-drug latencies to pain reaction.

A temperature of 2.5°C was used to assess potential antiallodynic properties of amitriptyline. In our present research this was the lowest temperature that had almost no effect on the pain sensitivity threshold of non-neuropathic mice. For this temperature, the mean latency time to pain reaction of mice before oxaliplatin injection was very close to the cut-off time in this assay, which clearly showed that at this temperature cold allodynia (i.e., pain response following normally non-painful thermal stimulation) could be assessed.

In our study oxaliplatin injection significantly reduced latency time to pain reaction in response to cold stimulation. Amitriptyline partially and dose-dependently reversed cold allodynia in neuropathic, oxaliplatin-treated mice. It is noteworthy that this effect was statistically significant only in the early phase and was noted only for doses of 2.5 mg/kg and 10 mg/kg. The antiallodynic effect of amitriptyline observed only in early-phase cold alldynia caused by oxaliplatin might suggest that Na⁺ protein expression underlies the development of cold alldynia in the early phase, but these channels are rather unlikely to play a key role in late-phase cold alldynia. As mentioned above, several recent studies have shown that early-phase cold alldynia after oxaliplatin administration can be attributed to the accumulation of its metabolite, oxalate, which acts by binding to axonal Na⁺ channels (Ewertz et al. 2015, Sakurai et al. 2009). Therefore, it seems plausible that in the early phase of neuropathy, after oxaliplatin administration, amitriptyline can compete with oxalate for binding to Na⁺ channels. Antiallodynic properties of amitriptyline were also investigated previously (Sada et al. 2012, Zhao et al. 2014). In a study by Sada et al. (2012), repeated administration of amitriptyline (5 and 10 mg/kg, oral route, once a day) reduced oxaliplatin-induced mechanical allodynia but not cold hyperalgesia in the acetone test in rats and reversed oxaliplatin-induced increases in the expression of NR2B protein and mRNA in rat spinal cord. Of note, in this study the acute effect of amitriptyline on animals’ pain behavior was not assessed.

The influence of amitriptyline on rapid-onset cold hypersensitivity as a main feature of the acute peripheral neuropathy induced by oxaliplatin in mice was also investigated by Zhao and colleagues (2014). In this experiment intraperitoneal amitriptyline (5 and 10 mg/kg) had no effect on cold hypersensitivity and the authors suggested that analgesics that directly suppress sensory neuron hyperexcitability (e.g., pregabalin, mexiletine) may be more efficacious than those which activate the descending pain inhibitory pathways (e.g., amitriptyline and opioids). Our results are not in line with those obtained in this study. A possible explanation for this difference is that these authors used a distinct mouse strain (C57BL/6J) and they assessed cold alldynia 2 h after oxaliplatin injection using the cold plate apparatus set at 5°C.

In conclusion, the present experiment confirmed that oxaliplatin is a potent inductor of cold hypersensitivity and is able to induce cold allodynia that can be detected at a relatively wide range of temperatures (1°C–4°C) that are not harmful to untreated, non-neuropathic subjects. Although various temperature ranges and different time-points of testing are being used in laboratories worldwide, in our study a rapid-onset cold alldynia in oxaliplatin-treated mice was shown for all temperatures tested. This effect was noted first 2 h after oxaliplatin injection for the temperature of 2.5°C. The present study also provides evidence for a potentially efficacious treatment for cold-triggered acute peripheral neuropathy induced by oxaliplatin with the use of amitriptyline. Amitriptyline at doses 2.5 and 10 mg/kg partially reversed early-phase cold alldynia caused by oxaliplatin but it was not effective in the late phase of neuropathy.

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REFERENCES


