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Interventional and preventive effects of aripiprazole and ceftriaxone used alone or in combination on oxaliplatin-induced tactile and cold allodynia in mice

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ABSTRACT

Background and purpose: Chemotherapy-induced peripheral neuropathy (CIPN) is a pharmacoresistant neurological complication induced by some antitumor drugs. This study aimed to assess antiallodynic properties of aripiprazole and ceftriaxone used alone or in combination to attenuate neuropathic pain related to CIPN caused by oxaliplatin.

Methods: Neuropathic pain was induced in mice by a single intraperitoneal dose of oxaliplatin (10 mg/kg). Aripiprazole and ceftriaxone were used in a single- or repeated dosing protocol. Their antiallodynic activity was assessed using von Frey and cold plate tests on the day of oxaliplatin injection and after 7 days. The influence of aripiprazole and ceftriaxone on animals' locomotor activity and motor coordination was also assessed.

Results: Single-dose and repeated-dose aripiprazole 10 mg/kg and ceftriaxone 200 mg/kg used alone and in combination attenuated early-phase and late-phase tactile allodynia in oxaliplatin-treated mice. Repeated administrations of ceftriaxone 200 mg/kg prevented the development of late-phase tactile allodynia. Both drugs showed no antiallodynic properties in the cold plate test. Single-dose aripiprazole 1 and 10 mg/kg but not its repeated administration significantly decreased locomotor activity of oxaliplatin-treated mice. Single-dose aripiprazole 1 and 10 mg/kg, aripiprazole 1 mg/kg + ceftriaxone 50 mg/kg and aripiprazole 1 mg/kg + ceftriaxone 200 mg/kg impaired motor coordination in the rotarod test.

Conclusions: In mice, neither ceftriaxone nor aripiprazole attenuated cold allodynia. Ceftriaxone alone could attenuate tactile allodynia caused by oxaliplatin without inducing motor adverse effects. Although the administration of aripiprazole reduced tactile allodynia, this effect seems to be limited considering severe motor deficits induced by this drug.

1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a non-fatal, severe treatment-related neurological complication resulting from the use of antitumor drugs such as vincristine, paclitaxel, platinum-based drugs, immunomoduators, epothilones, and bortezomib. It manifests mainly as sensory (painful) symptoms [1] and its treatment and prevention are not fully addressed [2].

Oxaliplatin is a third-generation platinum-based antineoplastic agent that alkylates DNA, thus inhibiting DNA synthesis [3]. Although its use in advanced colorectal tumors (in combination with 5-fluorouracil and folinate - FOLFOX) is well established, this therapy is often limited by concomitant adverse effects, among which CIPN, immunosuppressive activity and myelotoxicity are the most serious effects that are a frequent cause of therapy discontinuation or dose reduction [4]. CIPN induced by oxaliplatin significantly differs from that caused by other platinum-based drugs [4], *i.e.*, only oxaliplatin induces CIPN that manifests in two clinically distinct forms, namely acute and chronic. Acute neuropathy has not been reported for other platinum-based drugs [4–6] and is usually transient lasting for hours, often resolving within a few days [4,5]. It is observed in 86% of patients treated with oxaliplatin and is thought to be triggered by exposure to cold [5,6]. Cold-induced allodynia is also present in the chronic form of CIPN caused by oxaliplatin administration, and it is noteworthy that this chronic form of CIPN is featured by long-term peripheral sensory damage due to neuronopathy and it may worsen for several months

Abbreviations: CIPN, chemotherapy-induced peripheral neuropathy; CDT, combination drug therapy; GLT-1, glutamate transporter isoform 1

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following therapy discontinuation (described as "coasting" phenomenon) [6]. Numerous mechanisms are implicated in both forms of CIPN. It is thought that in the acute phase, no structural damage is present, and oxalate released from oxaliplatin causes chelation of calcium ions, thereby influencing ion channel functions, in particular voltage-gated sodium channel (Na_v) kinetics, whereas during the chronic phase accumulation of oxaliplatin in dorsal root ganglion cells leads to axonal degeneration [5,6].

The difficulties in the effective treatment and prevention of CIPN symptoms (*e.g.*, pain) have led to the introduction of combination drug therapy (CDT) as a method to alleviate pharmacoresistant neuropathic pain accompanying CIPN [7,8].

This concept is supported by several recent reports showing analgesic efficacy of combined pregabalin and tramadol in ovarian cancer patients on taxane-based therapy [7], combined dexmedetomidine and ulinastatin that reduced tactile allodynia in vincristine-treated rats [9], and the efficacy of pregabalin combined with ambroxol [10] but not cebranopadol combined with simvastatin [11] in oxaliplatin-treated neuropathic mice.

Apart from classical (*e.g.*, opioidergic) drug targets that are being investigated in the search for effective therapies for neuropathic pain in patients treated with CIPN-inducing antitumor drugs, other neurotransmission systems in the CNS have also become a subject of interest and extensive investigation for the discovery of more efficacious CIPN therapies. However, the idea to modulate pain by using drugs that affect neurotransmission systems is not novel, and this approach is confirmed by a successful application of antidepressant drugs as analgesic adjuvants in various neuropathic pain types. In this respect, antiallodynic and antihyperalgesic effectiveness of drugs enhancing serotoninergic and noradrenergic neurotransmission is very well established [12–14]. In contrast, less is known about the role of dopamine in pain modulation [15], although the available literature data indicate the effectiveness of some dopaminergic modulators (*e.g.*, duloxetine [16,17] or bupropion [18,19]) in the attenuation of pain, including CIPN-related pain [20–22].

The glutamatergic system also seems to be an attractive therapeutic target in terms of pain pharmacotherapy. Glutamate is a key neurotransmitter that participates in the enhancement of pain signal transmission and central sensitization of pain [23]. It has been reported that down-regulation of astrocytic glutamate transporter 1 (GLT-1) is responsible for the development of neuropathic pain [24,25], and it has been hypothesized that changes in the expression of spinal glutamate transporters are one of the critical factors in both the induction and maintenance of neuropathic pain [24]. Despite this widely known role of glutamate and its transporters in pain, only ketamine, an N-methyl D-aspartate receptor antagonist, has found a wider clinical application in the treatment of pain.

Our previous study showed antiallodynic efficacy of NLX-112, an agonist of serotonin 5-HT_{1A} receptors in a mouse model of neuropathic pain caused by oxaliplatin [12]. Also, it has been demonstrated previously that some atypical antipsychotics acting at serotonergic and dopaminergic receptors (*e.g.*, olanzapine, quetiapine, risperidone, ziprasidone and aripiprazole) attenuate several chronic pain conditions including diabetic neuropathy, post herpetic neuralgia, headache, musculoskeletal pain and fibromyalgia [26,27] but they have not been investigated in neuropathic pain related to CIPN.

Hence, in the present study, we used a mouse model of CIPN induced by oxaliplatin and assessed antiallodynic properties of aripiprazole – an antipsychotic drug acting as a partial agonist at dopamine D_2 and serotonin 5-HT_{1A} receptors and an antagonist at serotonin 5-HT_{2A} receptors [28], and ceftriaxone – a blood-brain barrier-penetrating, third-generation cephalosporin that acts to increase GLT-1 expression and function [25,29,30]. In our study, both aripiprazole and ceftriaxone were used alone or in combination to compare antiallodynic properties of (1) active doses of these drugs used in monotherapy, (2) inactive doses of these drugs used in monotherapy, and (3) combinations of active and inactive doses of these two drugs. Test drugs or their combinations were used in a single- or repeated (7-day) dosing protocols, and their antiallodynic activity was assessed in the early phase (*i.e.*, on the day of oxaliplatin injection) and the late phase (7 days after oxaliplatin administration) of CIPN. Antinociceptive properties of aripiprazole [28] and ceftriaxone [25,31] in neuropathic pain models have been reported recently however, none of these studies focused on the effect of these drugs on CIPN-related neuropathic pain. Moreover, none of these drugs were tested in a combination to alleviate CIPN-related neuropathic pain.

The number of drugs that effectively prevent CIPN development is very limited [21,32], and there is a paucity of reliable evidence for drugs recommended for CIPN prevention. In this regard, further research on these agents is warranted to find novel options for CIPN prevention [1]. Hence, as a part of this present study, we additionally aimed to establish, whether a repeated use of these drugs could prevent the development of persistent cold and tactile allodynia in mice treated with oxaliplatin.

2. Materials and methods

2.1. Animals and housing conditions

The experiments were performed at the Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow. The investigators involved in behavioral assays were blinded to the experimental groups to avoid potential bias in data recording. Adult male Albino Swiss (CD-1) mice weighing 18–22 g were purchased from the Animal Breeding Farm of the Jagiellonian University Faculty of Pharmacy. Before behavioral tests, the animals were kept in groups of 10 mice in standard plastic cages and housed under controlled conditions (room temperature of 22 ± 2 °C, light/dark (12:12) cycle, lights on at 8 A M, humidity 50 ± 10%, and free access to food and water). Experimental groups consisted of 6–10 animals/dose. For behavioral tests the animals were selected randomly, but the same animals were used throughout the two experimental sessions (*i.e.*, the acute phase and the late phase of oxaliplatin-induced neuropathy). Between these two sessions the mice were kept in standard laboratory conditions (described above). After the assay on the last day of experimentation (day 7), the mice were immediately euthanized by cervical dislocation. All experiments were performed between 9 A M and 2 PM. Experimental procedures for *in vivo* tests were approved by the Local Ethics Committee of the Jagiellonian University in Krakow (Approval No. 4/2016; 22.03.2016) and the treatment of animals was performed in full accordance with ethical standards laid down in respective Polish and EU regulations (Directive 2010/63/EU).

2.2. Chemicals

For *in vivo* tests, aripiprazole (Abilify 1 mg/mL, Otsuka, Japan) and ceftriaxone (Biotraxon, Polpharma S.A., Poland) were suspended in 0.9% saline (Polfa Kutno, Poland). Control animals received an appropriate amount of vehicle (0.9% saline). Oxaliplatin was purchased from Activate Scientific GmbH (Germany). To induce CIPN, oxaliplatin was freshly prepared by dissolving in 5% glucose solution (Polfa Kutno, Poland). The doses of aripiprazole, ceftriaxone, and oxaliplatin used in this study were chosen on the basis of our previous research [11,33] and available literature data [25,28,31,34].

2.3. Behavioral testing paradigm

A general description of the animal testing protocol and drug administration schedule are shown in Fig. 1. Briefly, naïve mice involved in the experiment were first tested for their sensitivity to mechanical (von Frey test) and cold (cold plate test) stimuli before oxaliplatin administration (referred to as paw withdrawal force or latencies "before

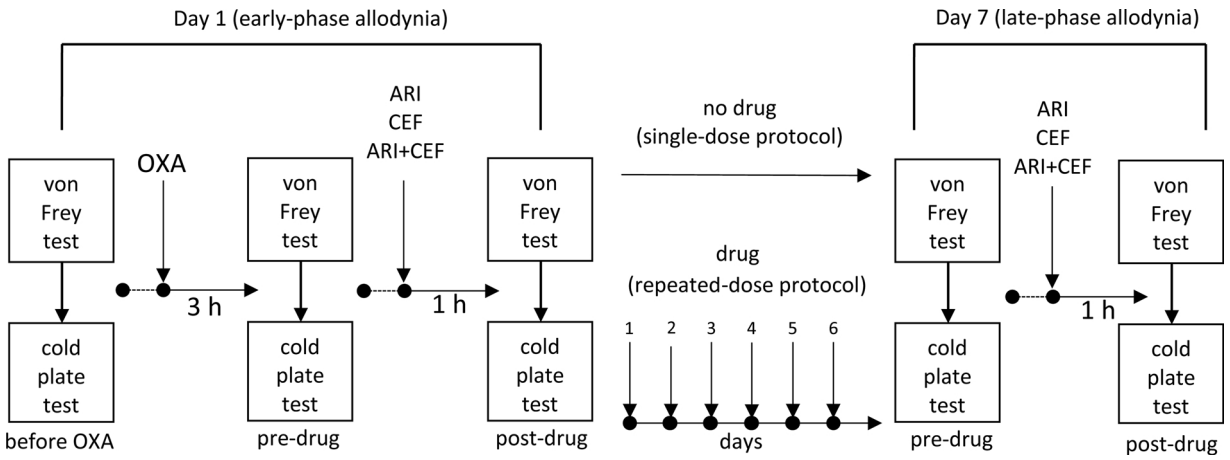

Fig. 1. Protocol used in the present study for the assessment of interventional and preventive properties of aripiprazole and ceftriaxone alone or in combination on tactile and cold allodynia caused by oxaliplatin administration. Tactile allodynia was measured using the von Frey test, while cold allodynia was assessed using the cold plate test. On day 1, the effect of oxaliplatin, aripiprazole, and ceftriaxone on early-phase allodynia was evaluated. On day 7, the effect of these agents on late-phase allodynia was assessed. Abbreviations: OXA – oxaliplatin; ARI – aripiprazole; CEF – ceftriaxone; before OXA – measurements obtained before oxaliplatin administration; pre-drug - measurements obtained before administration of aripiprazole, ceftriaxone; post-drug - measurements obtained 1 h after administration of aripiprazole and ceftriaxone.

oxaliplatin"). Then, the influence of oxaliplatin and test drugs on pain threshold was assessed in the early phase of allodynia. At this stage of the experiment, oxaliplatin was injected, and after 3 h, "pre-drug" measurements were obtained again with von Frey and cold plate tests. Aripiprazole and ceftriaxone alone or in combinations were then injected intraperitoneally, and after 1 h, "post-drug" measurements were obtained. This procedure, except for oxaliplatin injection, was repeated after 7 days to assess the effect of oxaliplatin, aripiprazole, and ceftriaxone on the late-phase allodynia in neuropathic mice. In the single-dose protocol, single doses of aripiprazole and ceftriaxone were used on day 1 and day 7 to establish their effect on the early and late phases of allodynia. In the repeated-dose protocol, test drugs were administered for 7 consecutive days, once daily, and the measurements of pain sensitivity were collected on days 1 and 7 (Fig. 1).

2.3.1. Induction of CIPN

CIPN was induced in mice by the intraperitoneal injection of a single dose of oxaliplatin (10 mg/kg) [33]. Pain sensitivity of neuropathic animals was measured in the cold plate test (assessment of cold allodynia - for details, please see Section 2.3.2) and in the von Frey test (assessment of tactile allodynia - for details, please see Section 2.3.3) performed on the day of oxaliplatin administration (referred to as "day 1") and after 7 days ("day 7") to establish the effect of aripiprazole and ceftriaxone, when used alone or in combination, on oxaliplatin-induced early-phase and late-phase allodynia, respectively.

2.3.2. Influence of aripiprazole and ceftriaxone on cold allodynia – cold plate test

The cold plate test was performed using the hot/cold plate apparatus (Bioseb, France) set at 2.5 °C. In this assay, the animals were tested first to obtain baseline latencies to pain reaction (*i.e.*, lifting, biting, shaking of hind paws, jumping and movement deficits) before oxaliplatin injection (referred to as latencies "before oxaliplatin"). Then, oxaliplatin was injected, and after 3 h, the latencies to pain reaction were measured again (referred to as early-phase allodynia "pre-drug latencies"). Next, test drugs were administered according to the protocol shown in Fig. 1, and early-phase allodynia post-drug latencies were measured. Pre-drug and post-drug latencies to pain reaction were measured again on day 7. At this time point, oxaliplatin was not administered. 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.

2.3.3. Influence of aripiprazole and ceftriaxone on tactile allodynia – von Frey test

The test was performed on day 1 and day 7 to assess the effect of aripiprazole and ceftriaxone used alone or in combination on tactile allodynia caused by oxaliplatin administration during the early-phase or late-phase sensory hypersensitivity. Mechanical hypersensitivity (tactile allodynia) in mice was assessed using the electronic von Frey unit (Bioseb, France) supplied with a single flexible filament applying increasing force (from 0 to 10 g) against the plantar surface of the hind paw of the mouse. The nocifensive paw withdrawal response automatically turned off the stimulus and the mechanical pressure that evoked the response was recorded. On the day of the experiment, the mice were placed individually in test compartments with a wire mesh bottom and were allowed to habituate for 1 h. After the habituation period, to obtain baseline (pre-drug) values, each mouse was tested 3 times alternately in each hind paw, allowing at least 30 s between each measurement. The mice were then pretreated with the test drugs or vehicle. After 60 min, the animals were tested again, and the mean post-drug values for each mouse were obtained [35].

2.3.4. Influence on locomotor activity

The locomotor activity test was performed using activity cages (40 cm x 40 cm x 30 cm, supplied with I.R. beam emitters) (Activity Cage 7441, Ugo Basile, Italy) connected to a counter for the recording of light-beam interrupts. Before the experiment, the mice were intraperitoneally pretreated with the test drugs or vehicle and then individually placed in the activity cages in a sound-attenuated room. The animals' movements (*i.e.*, the number of light-beam crossings) were counted during the next 30 min of the test. Before the experiment, the mice were habituated to activity cages for 15 min [36].

2.3.5. Influence on motor functions (rotarod test)

Before the test, the animals were trained for 3 consecutive days on the rotarod apparatus (Rotarod apparatus, May Commat RR0711, Turkey; rod diameter: 2 cm) that was rotated at a fixed speed of 18 rotations per minute (rpm). In each training session, the mice were placed on the rotating rod for 3 min with an unlimited number of trials. The proper experiment was performed 24 h after the last training session. After the administration of the test drugs or vehicle, the mice were tested on the rod that revolved at 6, 18, and 24 rpm. Motor impairments in mice were defined as the inability to remain on the rotarod apparatus for 1 min. The results are expressed as the mean time spent on the rotarod [36].

2.4. Data analysis

Data analysis was carried out using GraphPad Prism software (ver. 5.0, CA, USA). Numerical results are expressed as mean ± SEM. For statistical analysis, one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* comparison or two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison were used. $P < 0.05$ was considered significant.

3. Results

3.1. Effect of aripiprazole and ceftriaxone on cold allodynia – cold plate test

The cold plate test performed on days 1 and 7 after oxaliplatin administration aimed to establish (1) the effect of oxaliplatin on cold sensitivity threshold, (2) the effect of single-dose or repeated-dose aripiprazole and ceftriaxone, each used alone or in combinations on acute cold allodynia caused by oxaliplatin, (3) the effect of aripiprazole and ceftriaxone on late-phase cold allodynia, and (4) the ability of these drugs administered repeatedly after oxaliplatin injection and used alone or in combination to prevent the development of late-phase cold allodynia. The aims (2) and (3) investigated pharmacological activity of aripiprazole and ceftriaxone as interventional drugs, whereas aim (4) served to establish whether these drugs possess preventive properties in CIPN-related cold allodynia.

Statistical evaluation by two-way repeated measures ANOVA did not reveal an overall effect of treatment ($F[14,131] = 1.574$, $p = 0.0948$). Time affected the results significantly ($F[4,524] = 243.1$, $p < 0.0001$) and the interaction was also significant ($F[56,524] = 2.006$, $p < 0.0001$).

In both early and late phases of CIPN caused by oxaliplatin, significantly ($p < 0.0001$) decreased latency time to pain reaction was observed in all groups tested compared to values obtained before oxaliplatin administration (Fig. 2A, B). This implies that oxaliplatin lowered pain threshold for cold stimulation and induced cold allodynia in mice treated with this drug.

To assess whether aripiprazole and ceftriaxone used alone or in combination had antiallodynic properties, post-drug latencies in drug-treated and vehicle-treated neuropathic animals were compared. Statistically significant differences in post-drug latencies were observed during the early-phase cold allodynia between repeated-dose aripiprazole 10 mg/kg and vehicle + oxaliplatin-treated control mice ($p < 0.0001$) (Fig. 2A). Bonferroni's *post hoc* comparison also revealed a significant ($p < 0.05$ *vs.* post-drug latency of vehicle + oxaliplatin-treated mice) antiallodynic effect of repeated-dose aripiprazole 1 mg/kg in the late phase. Interestingly, in the late phase, such an effect was not observed for the dose 10 mg/kg (Fig. 2B).

A detailed analysis of changes within individual groups performed before and after treatment with aripiprazole revealed that in the early phase, repeated-dose aripiprazole 10 mg/kg increased latency time to pain reaction (significant at $p < 0.0001$ *vs.* pre-drug latency), but this was not observed in the late phase; similar results were obtained for repeated-dose aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg in the early phase ($p < 0.01$ *vs.* pre-drug latency), but not in the late phase of cold allodynia (Fig. 2A, B). Taken together, in the cold plate test, aripiprazole was found to be ineffective in alleviating cold allodynia in mice treated with oxaliplatin. Even if the dose 10 mg/kg prolonged the latency time to pain reaction in response to cold, this activity was transient and might rather be interpreted as an effect resulting from the reduced locomotor activity and impaired motor functions than its antiallodynic effect (for details, please see Sections 3.3,3.4 and Discussion).

In this test, ceftriaxone did not influence the latency time to pain reaction and was not able to attenuate cold allodynia induced by oxaliplatin, either in the early or late phase of neuropathy.

3.2. Influence of aripiprazole and ceftriaxone on tactile allodynia – von Frey test

The von Frey test performed on days 1 and 7 after oxaliplatin administration aimed to establish (1) the effect of oxaliplatin on mechanical nociceptive threshold, (2) the effect of single-dose or repeated-dose aripiprazole and ceftriaxone used alone or in combination on early-phase tactile allodynia caused by oxaliplatin, (3) the effect of aripiprazole and ceftriaxone on the late-phase tactile allodynia, (4) the ability of these drugs administered repeatedly after oxaliplatin injection and used alone or in combination to prevent the development of late-phase tactile allodynia. Similar to the cold plate test, aims (2) and (3) investigated pharmacological activity of aripiprazole and ceftriaxone used as interventional drugs, whereas aim (4) served to establish whether these drugs possess preventive properties for CIPN-related tactile allodynia.

Statistical evaluation of the results using two-way repeated-measures ANOVA revealed an overall effect of treatment ($F[10,95] = 14.03$, $p < 0.0001$). Time also affected the results significantly ($F[4,380] = 339.4$, $p < 0.0001$) and the interaction was significant ($F[40,380] = 7.843$, $p < 0.0001$).

A detailed analysis of changes within individual groups performed

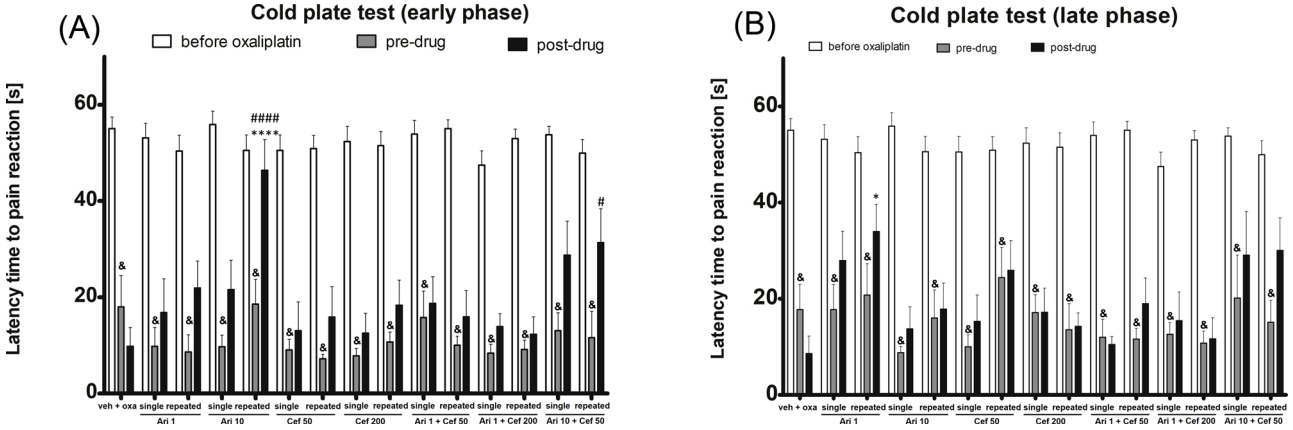

Fig. 2. Effect of aripiprazole and ceftriaxone used alone or in combination on cold allodynia measured in the cold plate test in oxaliplatin-treated mice. (A): effect on cold allodynia in the early phase of CIPN; (B): effect on cold allodynia in the late phase of CIPN. Results are shown as mean latency time to pain reaction (± SEM) for n = 8-10. Statistical analysis: repeated-measures ANOVA followed by Bonferroni's *post hoc* comparison. Significance: &: $p < 0.0001$ *vs.* latency before oxaliplatin; *: $p < 0.05$ *vs.* post-drug latency of oxaliplatin-treated control; ****: $p < 0.0001$ *vs*. post-drug latency of oxaliplatin-treated control; #: $p < 0.05$ *vs.*pre-drug latency; ####: $p < 0.0001$ *vs.*pre-drug latency.

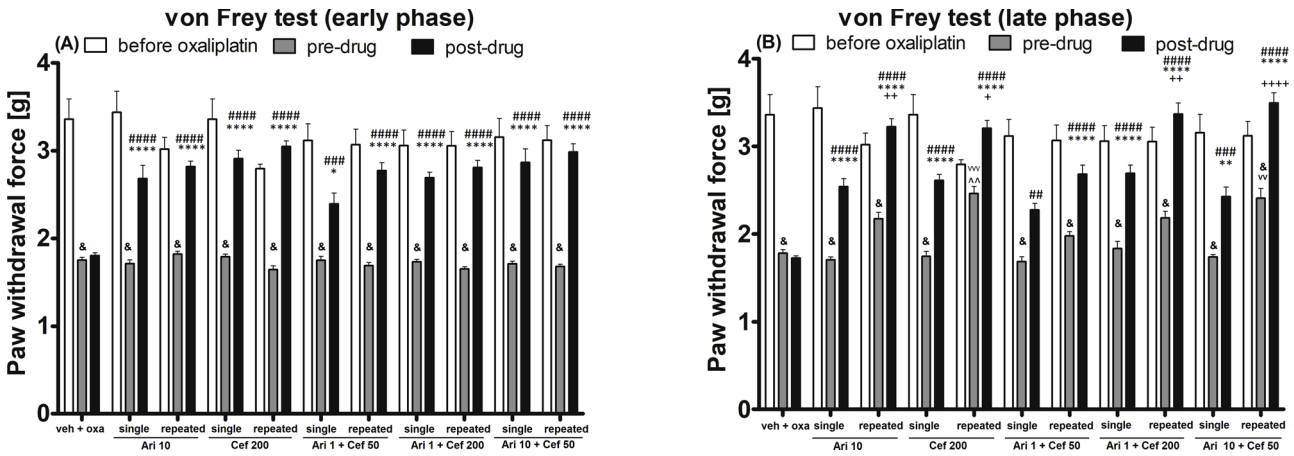

Fig. 3. Effect of aripiprazole and ceftriaxone used alone or in combination on tactile allodynia in oxaliplatin-treated neuropathic mice as measured in the von Frey test. (A): effect on tactile allodynia in the early phase of CIPN; (B): effect on tactile allodynia during the late phase of CIPN. Results are shown as mean paw withdrawal threshold (± SEM) for n = 8-10. Statistical analysis: repeated-measures ANOVA, followed by Bonferroni's *post hoc* comparison. Significance: &: $p < 0.0001$ *vs.* paw withdrawal threshold before oxaliplatin; *: $p < 0.05$ *vs.* post-drug paw withdrawal threshold of oxaliplatin-treated control; **: $p < 0.01$ *vs.* post-drug paw withdrawal threshold of oxaliplatin-treated control; ****: $p < 0.0001$ *vs.* post-drug paw withdrawal threshold of oxaliplatin-treated control; ##: $p < 0.01$ *vs.* pre-drug paw withdrawal threshold; ###: $p < 0.001$ *vs.* pre-drug paw withdrawal threshold; ####: $p < 0.0001$ *vs.* pre-drug paw withdrawal threshold; ^^: $p < 0.01$ *vs.* pre-drug paw withdrawal threshold of oxaliplatin-treated control; +: $p < 0.05$ *vs.* single-dose post-drug paw withdrawal threshold; + +: $p < 0.01$ *vs.* single-dose post-drug paw withdrawal threshold; + + + +: $p < 0.0001$ *vs.* single-dose post-drug paw withdrawal threshold; vv: $p < 0.01$ *vs.* single-dose pre-drug paw withdrawal threshold; vvv: $p < 0.001$ *vs.* single-dose pre-drug paw withdrawal threshold.

before and after treatment with oxaliplatin revealed that in the early phase of tactile allodynia (day 1) in all experimental groups, oxaliplatin significantly lowered pain threshold for mechanical stimulation ($p < 0.0001$ *vs.* paw withdrawal threshold before oxaliplatin injection). In contrast, on day 7, tactile allodynia was noted in all experimental groups ($p < 0.0001$ *vs.* value before oxaliplatin injection), except for the repeated-dose ceftriaxone 200 mg/kg group. This indicates that the 7-day administration of ceftriaxone 200 mg/kg attenuated the development of late-phase tactile allodynia in oxaliplatin-treated mice (Fig. 3). In this phase, the combination of repeated-dose aripiprazole 1 mg/kg and ceftriaxone 200 mg/kg also elevated pain threshold of oxaliplatin-treated mice, but in contrast to repeated-dose ceftriaxone 200 mg/kg alone, this effect did not reach statistical significance.

Compared to oxaliplatin-treated control mice, a significant elevation of post-drug paw withdrawal threshold was observed in both phases of neuropathy (Fig. 3A and B) in mice treated with single-dose and repeated-dose aripiprazole 10 mg/kg as well as in mice treated with single-dose and repeated-dose ceftriaxone 200 mg/kg (significant at $p < 0.0001$). For the combined single-dose aripiprazole 1 mg/kg + ceftriaxone 50 mg/kg group, the attenuation of tactile allodynia was noted only in the early phase ($p < 0.05$). Combined repeated-dose aripiprazole 1 mg/kg + ceftriaxone 50 mg/kg, and single-dose and repeated-dose aripiprazole 1 mg/kg + ceftriaxone 200 mg/kg attenuated tactile allodynia in both early and late phases of neuropathy (significant at $p < 0.0001$). For combined single-dose and repeated-dose administrations of aripiprazole 10 + ceftriaxone 50, the antiallodynic effect was also significant in both phases of tactile allodynia ($p < 0.01$).

During the late-phase tactile allodynia caused by oxaliplatin (Fig. 3B), statistically significant differences in post-drug paw withdrawal threshold were observed between single-dose and repeated-dose administration of aripiprazole 10 mg/kg ($p < 0.01$); ceftriaxone 200 mg/kg ($p < 0.05$); aripiprazole 1 mg/kg + ceftriaxone 200 mg/kg ($p < 0.01$); and aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg ($p < 0.0001$).

Interestingly, during the late-phase tactile allodynia induced by oxaliplatin, repeated administration of ceftriaxone 200 mg/kg significantly influenced pre-drug paw withdrawal threshold ($p < 0.01$ *vs.* oxaliplatin-treated control mice). In the late phase, there were also significant differences in pre-drug paw withdrawal threshold between single-dose and repeated-dose ceftriaxone 200 mg/kg ($p < 0.001$) as well as between single-dose and repeated-dose aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg ($p < 0.01$). Taken together, these findings suggest that repeated administration of ceftriaxone 200 mg/kg alone or aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg attenuated the development of late-phase tactile allodynia in oxaliplatin-treated mice (Fig. 3B).

In the von Frey test, a detailed comparison of pre-drug and post-drug values of paw withdrawal threshold within individual groups revealed a significant increase in the paw withdrawal threshold in both phases of oxaliplatin-induced tactile allodynia for single-dose and repeated-dose aripiprazole 10 mg/kg and ceftriaxone 200 mg/kg (significant at $p < 0.0001$) alone, and for single-dose and repeated-dose combination of aripiprazole 1 mg/kg + ceftriaxone 50 mg/kg (significant at $p < 0.01$), aripiprazole 1 mg/kg + ceftriaxone 200 mg/kg (significant at $p < 0.0001$), and aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg (significant at $p < 0.001$).

3.3. Influence on locomotor activity

In the locomotor activity test, an overall effect of treatment was observed ($F[16,72] = 13.55$; $p < 0.0001$). Time also affected the results significantly ($F[5,360] = 2.71$; $p < 0.05$), but treatment x time interaction was not significant ($F[80,360] = 1.03$; $p = 0.4117$). *Post hoc* analysis revealed no significant differences in animals' locomotor activity between non-neuropathic control mice and oxaliplatin-treated control mice at any time point of testing. In this assay, in oxaliplatin-treated control mice, there were no significant differences in the number of light-beam crossings measured on day of oxaliplatin administration and after 7 days.

In contrast, statistically significant differences in animals' locomotor activity were noted in mice treated with a single dose of aripiprazole 10 mg/kg and aripiprazole 1 mg/kg ($p < 0.05$ at all time points *vs.* oxaliplatin-treated control on the day of oxaliplatin administration; Fig. 4A). Interestingly, repeated administrations of aripiprazole 10 mg/kg and aripiprazole 1 mg/kg also decreased locomotor activity of mice but this effect (compared to oxaliplatin-treated control on the day of oxaliplatin administration) did not reach statistical significance.

Single or repeated administration of ceftriaxone at doses 50 and 200 mg/kg did not influence animals' locomotor activity (Fig. 4B). In contrast, various combinations of aripiprazole and ceftriaxone

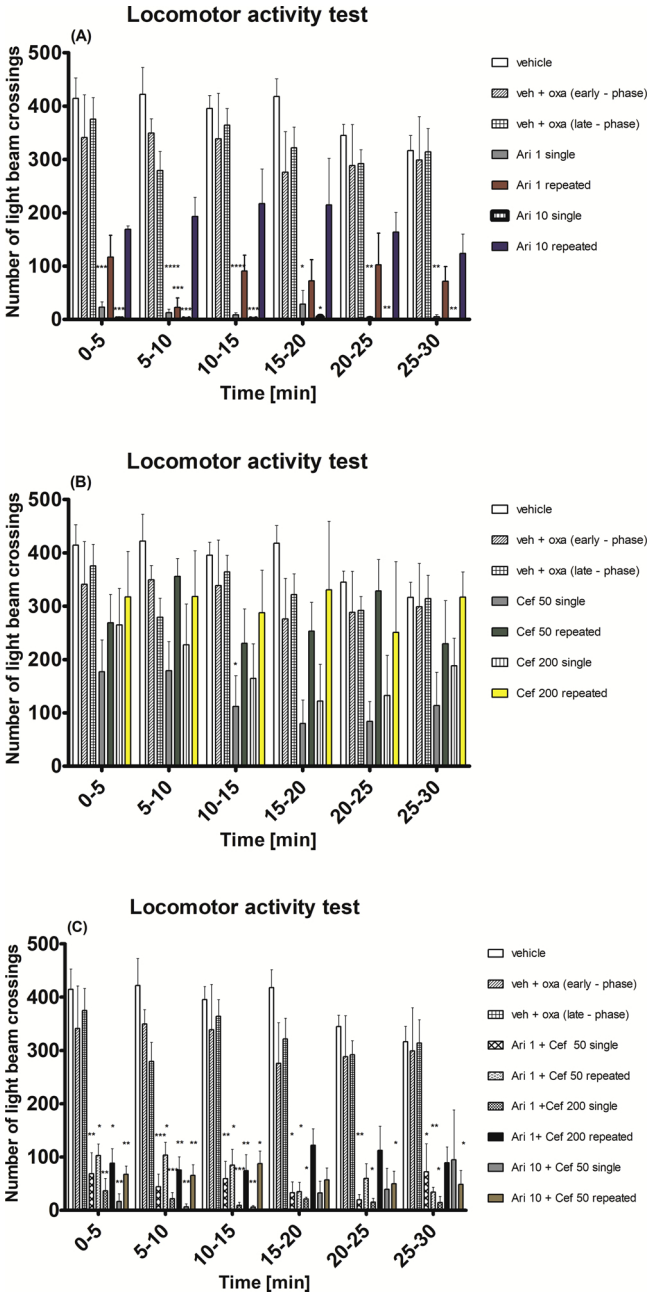

Fig. 4. Effect of aripiprazole alone (A), ceftriaxone alone (B), and aripiprazole and ceftriaxone used in combination (C) on animals' locomotor activity. Results are shown as mean number of light-beam crossings (± SEM) at selected time points for n = 6-8. Statistical analysis: repeated-measures ANOVA followed by Bonferroni's *post hoc* comparison. Significance *vs.* values of oxaliplatin-treated control measured on the day of oxaliplatin administration: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

significantly reduced locomotor activity of oxaliplatin-treated mice (Fig. 4C): single-dose aripiprazole 10 mg/kg co-administered with ceftriaxone 50 mg/kg (between 0–15 min: $p < 0.01$ *vs.* oxaliplatin-treated control on the day of oxaliplatin administration), repeated-dose aripiprazole 10 mg/kg co-administered with ceftriaxone 50 mg/kg (at all time points except for 15–20 min: $p < 0.05$ *vs.* oxaliplatin-treated control on the day of oxaliplatin administration), single-dose aripiprazole 1 mg/kg co-administered with ceftriaxone 200 mg/kg ($p < 0.05$ *vs.* oxaliplatin-treated control on the day of oxaliplatin administration), repeated-dose aripiprazole 1 mg/kg co-administered with ceftriaxone 200 mg/kg (between 0–15 min: $p < 0.05$ *vs.* oxaliplatin-treated control on the day of oxaliplatin administration), and single-dose and repeated-dose aripiprazole 1 mg/kg combined with ceftriaxone 50 mg/kg ($p < 0.05$ *vs.* oxaliplatin-treated control on the day of oxaliplatin administration).

3.4. Influence on motor functions (rotarod test)

In the rotarod test set at 6 rpm an overall effect of treatment was not observed ($F[15,80] = 1.276$, $p > 0.05$). At 18 and at 24 rpm, significant effects of treatment were noted (for 18 rpm: $F[15,80] = 3.721$, $p < 0.0001$, and for 24 rpm: $F[15,80] = 7.054$, $p < 0.0001$). As shown in Table 1, in the rotarod test, statistically significant differences were observed in the selected experimental groups.

4. Discussion

Recently, we have shown that the agonism at 5-HT_{1A} receptors attenuates some pain symptoms in a mouse model of oxaliplatin-induced neuropathic pain [12]. The main goal of the present study was to assess whether partial agonism at dopamine D_2 receptors combined with agonism at serotonin 5-HT_{1A} receptors or the decreased glutamate activity resulting from the increased expression of glutamate GLT-1 transporter might attenuate tactile and cold allodynia in CIPN-related neuropathic pain induced by oxaliplatin. For this purpose, aripiprazole – an atypical antipsychotic drug used for the treatment of some psychiatric disorders: schizophrenia and bipolar disorder, and ceftriaxone – a third-generation cephalosporin antibiotic, each used alone or in combination, were used, and these drugs were administered as single-dose or repeated-dose schedules. The study was, therefore, focused on the evaluation of these drugs' effects used both – as "intervention" and "prevention" protocols in CIPN-related neuropathic pain.

In mice, a single injection of oxaliplatin induced painful peripheral neuropathy accompanied by mechanical (tactile) and cold allodynia. These effects are widely described in the literature [37,38]. In our study, oxaliplatin lowered pain sensitivity threshold for both thermal and mechanical stimuli, and cold and tactile allodynia was observed as early as 3 h after oxaliplatin injection. This effect of oxaliplatin was persistent as it was also noted 7 days after its administration but interestingly not in all experimental groups. In the von Frey test, an increased pain threshold (*i.e.*, increased pre-drug paw withdrawal in the late phase) as compared to that of oxaliplatin-treated control mice and that of mice receiving single-dose ceftriaxone 200 mg/kg was noted in mice that were repeatedly injected with ceftriaxone 200 mg/kg. A similar effect was noted in the repeated-dose aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg group compared to mice treated with a single-dose combination of aripiprazole 10 mg/kg + ceftriaxone 50 mg/kg. Importantly, in the repeated-dose ceftriaxone 200 mg/kg group, in the late phase of tactile allodynia, the pre-drug paw withdrawal force was not significantly different from that measured before oxaliplatin injection. This indicates that the 7-day administration of ceftriaxone 200 mg/kg inhibited or prevented from the development of tactile allodynia. This finding demonstrates a potential utility of this drug in the prevention of development of late-phase tactile allodynia caused by oxaliplatin. However, it should be noted here that the 7-day administration of ceftriaxone 200 mg/kg had no effect on cold allodynia in CIPN and this seems to be the main limitation for the use of ceftriaxone in CIPN-related cold hypersensitivity. Prevention of CIPN is regarded as a significant and unresolved medical problem worldwide, and currently no definite therapies for prevention are established. Therefore, it is of key importance to search for novel preventive strategies for CIPN as many drugs approved for the interventional use in CIPN-related pain completely failed or showed limited preventive effect on CIPN development [21,32]. Previously, it was demonstrated that intraperitoneally administered ceftriaxone had both preventive and therapeutic properties in a chronic constriction injury (CCI) model of neuropathic pain in rats. In that study, ceftriaxone prevented the development and reversed

Table 1
Effect of aripiprazole alone, ceftriaxone alone, aripiprazole and ceftriaxone used in combination, on animals' motor coordination measured using the rotarod test.

Treatment [mg/kg]	Time on the rotarod [s] ± SEM (6 rpm)	Time on the rotarod [s] ± SEM (18 rpm)	Time on the rotarod [s] ± SEM (24 rpm)
Vehicle	54.2 ± 4.1	60.0 ± 0.0	60.0 ± 0.0
Vehicle + oxaliplatin	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0
Aripiprazole 1 (single-dose)	60.0 ± 0.0	51.0 ± 3.5	19.3 ± 6.8 **
Aripiprazole 1 (repeated-dose)	51.4 ± 7.3	54.1 ± 5.9	47.8 ± 8.1
Aripiprazole 10 (single-dose)	44.8 ± 5.3	11.3 ± 7.6 ***	13.5 ± 7.1 ***
Aripiprazole 10 (repeated-dose)	60.0 ± 0.0	54.5 ± 5.0	60.0 ± 0.0
Ceftriaxone 50 (single-dose)	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0
Ceftriaxone 50 (repeated-dose)	57.5 ± 2.5	60.0 ± 0.0	60.0 ± 0.0
Ceftriaxone 200 (single-dose)	57.8 ± 2.3	60.0 ± 0.0	56.3 ± 3.8
Ceftriaxone 200 (repeated-dose)	60.0 ± 0.0	60.0 ± 0.0	60.0 ± 0.0
Aripiprazole 1 + Ceftriaxone 50 (single-dose)	59.3 ± 0.8	40.5 ± 8.2	28.3 ± 11.0 *
Aripiprazole 1 + Ceftriaxone 50 (repeated-dose)	60.0 ± 0.0	42.5 ± 13.5	47.0 ± 11.4
Aripiprazole 1 + Ceftriaxone 200 (single-dose)	60.0 ± 0.0	41.0 ± 12.6	22.8 ± 13.2 **
Aripiprazole 1 + Ceftriaxone 200 (repeated-dose)	60.0 ± 0.0	60.0 ± 0.0	47.8 ± 7.1
Aripiprazole 10 + Ceftriaxone 50 (single-dose)	60.0 ± 0.0	42.5 ± 13.5	34.3 ± 9.0
Aripiprazole 10 + Ceftriaxone 50 (repeated-dose)	59.7 ± 0.3	46.5 ± 7.1	36.1 ± 7.0

Results are shown as mean time spent on the rotarod (± SEM) revolving at 6 rpm, 18 rpm and 24 rpm for n = 6-8. Statistical analysis: one-way ANOVA, followed by Dunnett's *post hoc* comparison. Significance *vs.* oxaliplatin-treated control on the day of oxaliplatin administration: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

tactile allodynia and heat hyperalgesia, and this activity was accompanied by an increased spinal expression of GLT-1 (EAAT2) transporter [25]. GLT-1 is the main glutamate transporter in rats and is responsible for the removal of 90% of extracellular glutamate in the CNS [39]. Many lines of evidence show its involvement in the development and maintenance of acute and chronic pain [25,40]. By upregulating GLT-1 expression, ceftriaxone increased glutamate reuptake in the CNS [30,41,42] and alleviated pain by reducing spinal astrocyte activation and neuronal hyperexcitability [41,42]. A lowered GLT-1 expression was found to play an important role in oxaliplatin-induced neuropathic pain. In a study performed by Yamamoto and colleagues [43], oxaliplatin disrupted the extracellular glutamate homeostasis in the spinal cord, thus resulting in neuropathic symptoms in rats. *In vivo* spinal microdialysis revealed that the baseline glutamate concentration was elevated in oxaliplatin-treated rats, and that mechanical stimulation of the hind paw significantly increased extracellular glutamate concentration. In these rats, the expression of GLT-1 was decreased in the spinal cord. Taken together, these data indicate that increasing spinal GLT-1 expression might alleviate neuropathic pain. These findings could be extrapolated to those obtained in our present study. In line with this, in previous studies, downregulation of GLT-1 has been linked with the use of taxanes [44,45]. The effect of ceftriaxone on GLT-1 expression was also confirmed in a study performed by Hajhashemi et al. [46] who tested a 7-day administration of intraperitoneal ceftriaxone 100–400 mg/kg and noted reduced allodynia and hyperalgesia after treatment. This beneficial effect was persistent mainly for the dose of 200 mg/kg twice daily. In the context of our present study, it should be noted that in this previous research, one daily dose of ceftriaxone 200 mg/kg only partially attenuated tactile allodynia in CCI rats and only slightly reduced cold allodynia. This finding proved that in CCI rats, ceftriaxone affected mechanical and to a lesser degree thermal (cold) allodynia, suggesting that the biological functions of GLT-1 are more associated with the regulation of mechanical nociceptive threshold than cold nociceptive threshold [46]. This result is in line with our present study showing that ceftriaxone 200 mg/kg administered once daily, even for 7 days, had no significant effect on cold allodynia caused by oxaliplatin, but it reduced the development of tactile allodynia. Another study also showed that the effect of ceftriaxone on cold allodynia in CCI rats was transient, and cold allodynia was less attenuated than mechanical allodynia [31]. At the same time, the effect of ceftriaxone was enhanced by a co-administration with minocycline [31]. Our previous studies also revealed that in a CIPN model in mice, higher dose regimens of analgesic drugs are necessary for the attenuation of cold allodynia than those effectively reducing tactile allodynia [10,33]. A potential explanation for this observation is that both cold and mechanical stimuli can activate distinct types of nociceptors that are differentially sensitive to these stimulators [47,48].

The results of the present study confirmed that ceftriaxone used as an intervention did not reduce cold allodynia in neuropathic mice, but it effectively attenuated tactile allodynia in the von Frey test in both phases of oxaliplatin-induced neuropathy. Similar to our finding showing that the repeated administration of ceftriaxone 200 mg/kg had a stronger (than a single-dose ceftriaxone 200 mg/kg) impact on the reversal of tactile allodynia, in the visceral pain model, significant and dose-dependent antinociception of ceftriaxone (25–200 mg/kg per day) was observed after the 7-day pretreatment. This effect was higher after a 7-day pretreatment than after acute administration [34]. Antiallodynic properties of ceftriaxone were also enhanced by co-administration with minocycline [31]. This effect was found to be persistent even at low dose ceftriaxone 100 mg/kg + minocycline 50 mg/kg after a 7-day treatment, but it was most enhanced in the ceftriaxone 200 mg/kg + minocycline 50 mg/kg group [31]. The combinations of ceftriaxone and nonsteroidal analgesics or levetiracetam also demonstrated synergistic action between these drugs in rodent pain models. These results suggest that such combination drug therapies might be useful in the treatment of inflammation-related pain [34].

Not only GLT-1 proteins but also striatal dopamine D_2 receptors and dopaminergic pathways from the ventral tegmental area and nucleus accumbens are thought to be involved in the regulation of pain in humans and experimental animals [49]. Positron emission tomography technique showed that dopamine D_2 receptor availability in the striatum of healthy subjects was associated with the regulation of cold pain threshold [50]. Furthermore, a key role of the biogenic amine system and single nucleotide polymorphisms in serotonin and dopamine transporter and receptor genes has been identified in fibromyalgia syndrome. This resulted in altered heat, cold, and mechanical sensitivity due to impairment in the ascending and descending somatosensory systems and decreased CNS inhibition of peripheral nociceptive signaling [51]. Higher extracellular dopamine levels and reduced expression of D_2, but not D_1, receptors in the nucleus accumbens were noted as a possible maladaptation effect in response to chronic pain and peripheral neuropathy [52].

Intraperitoneal aripiprazole (0.1–10 mg/kg) attenuated pain responses in a mouse formalin test and diminished PGE_2-induced hyperalgesia in the paw pressure test. This effect was reversed by D_2, 5-HT_{1A} and δ-opioid receptor antagonists, suggesting the activation of dopaminergic, serotonergic and δ-opioid receptors [28,53] as a possible mechanism underlying antinociception. Therefore, it was suggested

that aripiprazole and other dopaminergic modulators should be considered as potential new treatments for certain pain types [28].

Recently, a strong link has been demonstrated between glutamatergic, dopaminergic and opioidergic systems in the attenuation of CCI neuropathic pain [55]. Moreover, ceftriaxone affected dopaminergic transmission, and in a rat model of Parkinson's disease, it showed neuroprotective properties [56]. It has also been shown that acute administration of ceftriaxone attenuated allodynia by activation of dopaminergic and opioidergic pathways [55]. Taken together, these data indicate an important link between glutamatergic and dopaminergic neurotransmission systems in the perception of painful stimuli and this was the rationale for investigating the activity of ceftriaxone and aripiprazole, each used alone or in combination in this present study.

In our research, in the cold plate test, aripiprazole alone was not generally effective, but it attenuated pain responses in the von Frey test. This effect for single-dose administration protocols for aripiprazole should, however, be interpreted cautiously considering the results obtained in the locomotor activity and rotarod tests. Both these assays were used for a proper interpretation of data obtained in pain tests to avoid the possibility of false positive results [54], and they showed that single-dose aripiprazole 10 mg/kg significantly impaired motor skills of experimental animals. Such deficits were not noted for groups treated repeatedly with aripiprazole; this finding indicated that after repeated dosing, mice became habituated to this negative impact of aripiprazole. It should be clearly stated that aripiprazole-induced decreased locomotor acitivity is a significant limitation of the potential use of this drug in neuropathic pain conditions, as it may worsen daily functioning of patients. In addition, this effect may be potentiated by adverse effects of this drug on motor functions demonstrated in the rotarod test.

In our present study we also tested combinations of aripiprazole + ceftriaxone for their ability to attenuate allodynia caused by oxaliplatin. Significant reduction of early-phase and late-phase tactile allodynia was noted for all aripiprazole + ceftriaxone combinations tested. Here, these results should again be interpreted cautiously considering significantly reduced locomotor activity after the administration of all combinations tested and motor coordination deficits observed in the rotarod test for single-dose combinations of aripiprazole 1 mg/kg and ceftriaxone 50 mg/kg, or aripiprazole 1 mg/kg and ceftriaxone 200 mg/kg. The mechanism underlying these observed *in vivo* effects remains to be fully elucidated. A potential pharmacokinetic interaction between ceftriaxone and aripiprazole should be taken into consideration. Metabolic interactions between these two drugs should rather be excluded, considering that a large proportion of ceftriaxone is excreted as unchanged drug [57], while aripirazole is not either cytochrome P450 inducer or inhibitor. A pharmacokinetic drug-drug interaction at the blood brain barrier resulting in an increased drug distribution might be responsible for the observed adverse effects of combined aripiprazole and ceftriaxone because ceftriaxone is a blood-brain barrier-permeating agent and aripiprazole also acts at the CNS level.

5. Conclusions

The present study demonstrated potential utility of ceftriaxone in the attenuation of tactile allodynia but not cold allodynia in neuropathic, oxaliplatin-treated mice. In contrast, apparently beneficial results obtained for aripiprazole raise some doubts, considering that they may be false positives resulting from the adverse effects of this drug on the motor functions of experimental animals. Much concern is also given to the combined use of both drugs in the view of potential adverse effects (motor deficits and reduced locomotor activity) observed for aripiprazole and ceftriaxone used as CDT. Also, the lack of antiallodynic activity of ceftriaxone and aripiprazole to attenuate cold allodynia seems to be a serious limitation for using these drugs in neuropathic pain caused by oxaliplatin. An important finding of this present research is that repeated administrations of ceftriaxone 200 mg/kg attenuate the development of tactile allodynia in oxaliplatin-treated mice.

It should be emphasized here that although the overuse of antibiotics (*e.g.*, ceftriaxone) for non-infectious diseases might lead to microbial resistance, a reasonable application of SAR studies can provide compounds with no antimicrobial effects but still active at GLT-1 [58]. On the basis of the present results and data from other laboratories, such compounds would be of immense value in the prevention and treatment of neuropathic pain of various origins. The findings of this and previous studies propose that ceftriaxone can be included in treatment protocols of neuropathic pain (and possibly other glutamate-dependent diseases). In contrast, partial agonists of dopaminergic receptors, such as aripiprazole, do not seem to be useful in the treatment of neuropathic pain induced by antitumor drugs.

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Declaration of interest

The authors declare no conflict of interest.

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