

# Characterization of cannabinoid-induced relief of neuropathic pain in a rat model of cisplatin-induced neuropathy

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## ABSTRACT

Clinical use of antineoplastic drugs is associated with the development of numerous adverse effects that many patients find intolerable, including peripheral neuropathy. Cannabinoids have relieved neuropathic pain in different animal models. But their therapeutic activities could be affected by their psychoactive properties.

The aim of this work was to determine the effect of cannabinoids in cisplatin-evoked neuropathy. For this purpose, the non-selective agonist WIN 55,212-2 (WIN), the CB1-selective agonist ACEA or the CB2-selective agonist JWH133 (or their vehicle) was either systemically administered at a non-psychoactive dose or locally injected in cisplatin-treated rats. Selective CB1 and CB2 cannabinoid antagonists (AM251 and SR144528, respectively) were used to characterize cannabinoid effects.

Cisplatin-treated rats showed mechanical allodynia but not thermal hyperalgesia. Cannabinoid agonists alleviated mechanical allodynia. This effect was mediated by both CB1 and CB2 cannabinoid receptors when the cannabinoid was systemically applied. At the dose used, cannabinoid agonists had no psychoactive effect. The local effect of the drug involved the activation of peripheral CB1 receptors whereas involvement of CB2 receptors was less clear.

In a rat model of cisplatin-induced neuropathy, cannabinoids have an antinociceptive effect, but the cannabinoid receptors involved could be different depending on the route of administration. Non-psychoactive doses of cannabinoid agonists are capable of alleviating the signs of peripheral neuropathy when systemically applied. Interestingly, local administration of selective CB1 agonists or systemic administration of CB2 agonists, which are non-psychoactive, may serve as new therapeutic alternatives for symptom management in painful neuropathy associated with cisplatin treatment.

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## 1. Introduction

Peripheral neuropathy is one of the major adverse effects of chemotherapy (Windebank and Grisold, 2008; Stillman and Cata, 2006; Markman, 2006) and not only reduces the quality of life, but the development of neuropathic symptoms may also demand premature cessation of treatment. The major classes of antineoplastic agents, including the vinca alkaloids (e.g. vincristine), taxane (e.g. paclitaxel) and platinum-derived (e.g. cisplatin) compounds, are associated with the development of neuropathic pain. Specifically, cisplatin induces a duration-, dose-, and time-dependent axonal sensorimotor polyneuropathy affecting large and small diameter sensory fibers. Cisplatin neurotoxicity is predominantly characterized by sensory neuropathy with initial complaints of pain and paresthesias in the distal extremities (Ta et al., 2009). Up to 30–40% of cancer patients that receive this agent experience pain (Khasabova et al., 2012) and about 20% of patients are unable to complete a full course of cisplatin therapy due to sensory neuropathy. Many agents have been proposed to manage

chemotherapy-induced neuropathy (acetylcysteine, amifostine, calcium and magnesium, diethyldithiocarbamate, glutathione, or vitamin E), but to date, the data are insufficient to conclude that any of the purported agents prevent or limit the neurotoxicity of platinum drugs among human patients (Albers et al., 2011). The absence of effective treatments for chemotherapy-evoked neuropathy makes the identification of alternative analgesics a crucial medical need.

The cannabinoid system is one of the endogenous systems that modulate pain perception. In fact, cannabinoids have traditionally been used for the treatment and/or prevention of chemotherapy side-effects. In experimental models, not only the non-selective CB1/CB2 agonist WIN55,212-2 (WIN) suppressed neuropathic nociception induced by paclitaxel through a CB1-specific mechanism (Pascual et al., 2005) but also CB2 selective agonists attenuated neuropathy (Rahn et al., 2008). Likewise WIN suppressed vincristine-induced neuropathy through the activation of both CB1 and CB2 receptors (Rahn et al., 2007). Previously, we have seen that WIN prevented the development of mechanical allodynia in cisplatin- (Vera et al., 2007) and paclitaxel- (Burgos et al., 2012) treated rats. Although cannabinoids might also exert acute antinociceptive effects in cisplatin-induced neuropathy, this has not been tested so far.

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This potentially useful antinociceptive/analgesic effect of cannabinoids could be affected by their psychoactive activity, mediated by CB1 receptors expressed in the central nervous system (CNS). Upon topical application, cannabinoids have reduced pain in a human experimental model (Rukwied et al., 2003). In animal models, local administration of CB1 receptor agonists produced anti-nociceptive effects in both inflammatory and neuropathic conditions (Fox et al., 2001; Nackley et al., 2003; Richardson et al., 1998; Vera et al., 2012). Therefore, the activation of peripheral CB1 receptors (Karst and Wippermann, 2009) or the use of CB2 agonists, devoid of central effects, might be good alternatives for neuropathy management.

So, the aims of this work were to determine: 1. the acute effect of cannabinoids on cisplatin-evoked neuropathy in the rat, 2. the psychoactive effects of cannabinoids at the dose tested in neuropathic animals, and 3. the involvement of CB1 and CB2 receptors in the antinociceptive activity of cannabinoids systemically or locally applied.

## 2. Methods

The experiments, which were designed to minimize the number of animals used and their suffering, were performed in strict accordance with the EU directive for the protection of animals used for scientific purpose (2010/63/UE) and were approved by the Ethical Committee at the Universidad Rey Juan Carlos.

### 2.1. Animals

Male Wistar rats (250–300 g) obtained from the Veterinary Unit of Universidad Rey Juan Carlos were used for all experiments. Animals were housed, grouped (4–6/cage), in standard transparent cages (60 × 40 × 20 cm) that were furnished with wood shaving bedding, which was changed every 1–2 days. Cages were placed adjacent to each other under environmentally controlled conditions (temperature = 20 °C; humidity = 60%) with a 12 h light/12 h dark cycle (lights on between 08:00 and 20:00 h). Animals had free access to standard laboratory rat chow (Harlan Laboratories) and tap water. Experiments started at least one week after arrival of animals to the laboratory.

### 2.2. Induction of neuropathy

During the first week (W0), rats were habituated to the testing procedures and to handling by the investigator. After this period of adaptation, rats received one intraperitoneal (*i.p.*) injection of either cisplatin (at 2 mg/kg) or saline (0.9% w/v, 1 mL/kg), once per week for five weeks (W1–W5), on the first day of each experimental week. In order to prevent eventual nephrotoxicity induced by chronically administered cisplatin, 2 mL of saline was also injected subcutaneously just before intraperitoneal saline or cisplatin administration (Authier et al., 2003).

### 2.3. Evaluation of overall health and neuropathy

All rats were regularly examined throughout the experiment in order to detect signs of general toxicity: aggressiveness, difficulties in handling, piloerection, vocalization while being handled and diarrheas.

The development of peripheral nociceptive neuropathy was evaluated using tests for both mechanical allodynia and heat-hypo/hyperalgesia at the beginning of the experiment (W0) and 4 days after the last administration (W5). An observer unaware of the treatments recorded the test values.

For mechanical sensitivity, rats were placed individually on an elevated iron mesh in a clear plastic cage and were allowed to adapt to the testing environment for at least 10 min. Habituation to this environment was also performed two days before assessment. Mechanical allodynia was assessed using an electronic Von Frey apparatus

(EVF3, Bioseb, BP89, Chaville Cedex, France). The Von Frey test was applied to the plantar surface of each hindpaw, through the mesh floor. The test was performed four times with an interstimulus interval of approximately 30 s. The mean of the four trials was used for data analysis. Mechanical allodynia was defined as a significant decrease in Von Frey Hairs withdrawal threshold evoked by mechanical stimuli. The apparatus has an upper cut-off limit for testing of 50 g.

Responses to thermal stimuli were evaluated right after mechanical allodynia, using a 37370 plantar test apparatus (Ugo Basile, Comerio VA, Italy). The withdrawal latency from a focused beam of radiant heat applied to the mid plantar surface of the hindpaws was recorded. The intensity of the light was adjusted at the beginning of the experiment so that the control average baseline latencies were about 8 s and a cut-off latency of 25 s was imposed. The withdrawal latency of each paw was measured during three trials separated by 2 min intervals, and the mean of the three readings was used for data analysis.

### 2.4. Effect of acute intraperitoneal administration of cannabinoids on mechanical and thermal sensitivity

Four days after the last cisplatin administration, right after neuropathy assessment, three sets of experiments were carried out in cisplatin-treated or control (saline-treated) rats to test and characterize the effect of cannabinoids systemically administered. First, a single dose of vehicle (1 mL/kg) or the non-selective cannabinoid agonist WIN (1 mg/kg) was intraperitoneally administered. This dose was selected based on previous research from ours and other laboratories (Vera et al., 2007, 2012; Pascual et al., 2005; Bujalska, 2008). Second, to characterize the implication of CB1 and CB2 receptors, some rats received an *i.p.* injection of the CB1 or the CB2 antagonists (AM251 or SR144528; 1 mg/kg in each case) or both, 20 min prior to WIN/vehicle *i.p.* injection. And third, some cisplatin-treated animals received an injection of the CB1 (ACEA) or CB2 (JWH133) selective agonists (1 mg/kg in each case) with or without the previous administration of the corresponding antagonist (AM251 and SR144528, 1 mg/kg).

### 2.5. Central effects of cannabinoids intraperitoneally administered (cannabinoid tetrad)

The classical cannabinoid tetrad test was recorded in the animals after cannabinoid *i.p.* administration to monitor the central effects at the dose tested. To check for central actions of cannabinoids, cisplatin-treated rats received one intraperitoneal injection of vehicle ( $n = 8$ ), WIN ( $n = 8$ ), ACEA ( $n = 8$ ) or JWH133 ( $n = 8$ ) at 1 mg/kg and the cannabinoid tetrad was subsequently assessed. For comparison, the central effects of the cannabinoid drugs were also tested in naïve rats. As a positive control, WIN was used at 5 mg/kg ( $n = 6$ ) which is a dose that had previously produced central effects in our hands (Abalo et al., 2011).

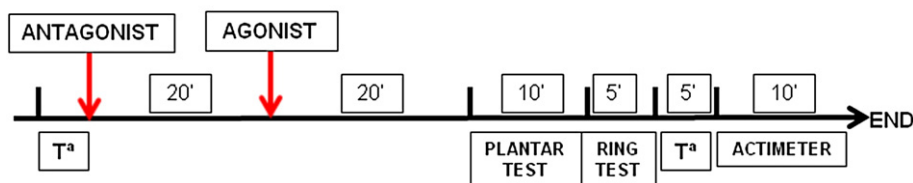
Cannabinoid tetrad evaluates antinociception (thermal sensitivity), rectal temperature, catalepsy and spontaneous locomotor activity (Compton et al., 1993). Parameters were recorded as shown in Fig. 1, by an observer unaware of the treatments, as previously reported (Abalo et al., 2009, 2010; Vera et al., 2012).

Thermal sensitivity was measured using the plantar test 20 min after drug administration as described above.

Core temperatures were measured using a P6 thermometer and a lubricated rectal probe (Cibertec, Spain) was inserted into the rectum to a constant depth of 5 cm. Data were recorded both before drug administration and 30 min after injection.

To measure catalepsy rats were hung by their front paws from a rubber coated metal ring (12 cm diameter) fixed horizontally at a height allowing their hindpaws to just touch the bench, and the time taken for the rat to move off the ring was measured with a cut-off of 30 s. Data are expressed as an immobility index defined as percentage of the total time spent on the ring during which the animal remains

## CANNABINOID TETRAD



**Fig. 1.** Protocol for the performance of the cannabinoid tetrad. The sequence of tests and temporal intervals of them during the cannabinoid tetrad performed to assess central effects of cannabinoid agonists are shown. Cannabinoid antagonists (or vehicle) are administered after basal core temperature recording ( $T_a$ ), and 20 min prior to cannabinoid agonists (or vehicle). The cannabinoid tetrad is performed 20 min after cannabinoid agonist (or vehicle) administration, in the following order: plantar test (for analgesia), ring test (for catalepsy), core temperature ( $T_r$ , for hyperthermia), and actimeter (for hypolocomotion).

motionless. Latencies were measured after temperature evaluation, 35 min after drug or vehicle administration.

Spontaneous locomotor activity was evaluated using individual photocell activity chambers (Cibertec, Spain). Rats were placed in the recording chambers ( $55 \times 40$  cm, with a 3 cm spacing between beams) 40 min after drug administration, and the number of interruptions of photocell beams was recorded over a 10-min period. The mean number of crossings of the photocell beams was used for comparison.

### 2.6. Effect of cannabinoids locally administered

All the cannabinoid agonists (WIN, ACEA or JWH133) and antagonists (AM251 or SR144528) used in the experiments previously described were intraplantarly (i.pl.) administered to characterize the antinociceptive effect of the cannabinoids locally applied. In this case the dose was  $50 \mu\text{g}$  in  $25 \mu\text{l}$  of vehicle or  $25 \mu\text{l}$  of vehicle alone. The cannabinoid agonists were administered 20 min prior to the tests. When the antagonists were used, these were administered 20 min prior to the agonist (that is, 40 min prior to the tests). The injection took place in the right hindpaw and the left paw served as control.

### 2.7. Compounds and drugs

WIN 55,212-2, ACEA and JWH133 were obtained from Tocris Cookson (Bristol, U.K.) and AM251 was purchased from Ascent Scientific Ltd. (North Somerset, BS24 9 ES, UK). SR144528 was kindly gifted by Sanofi Aventis Recherche & Developpement (Montpellier, France). All cannabinoid agonists and antagonists were dissolved in Tocrisolve (Tocris, Cookson, Bristol, UK). Cisplatin was purchased from Sigma Aldrich (Spain) and was dissolved in saline (sonicated for about 15 min).

### 2.8. Statistical analysis

All data were expressed as the mean values  $\pm$  the standard error of the mean (SEM). Intra-group differences were analyzed using Student's t-test. Differences between groups were analyzed using one-way ANOVA followed by the *post hoc* Bonferroni's test for multiple comparisons. All analyses were performed with Prisma statistical package (version 4.0). Values of  $p < 0.05$  were regarded as being significantly different.

## 3. Results

### 3.1. Development of peripheral neuropathy

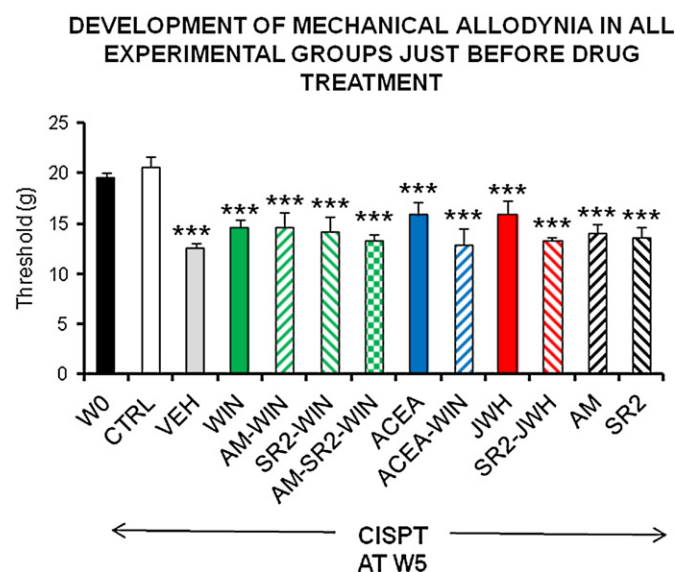
The threshold for mechanical sensitivity before treatment (W0) was  $19.49 \pm 0.51$  g ( $n = 40$ ). In control, saline-treated rats, this threshold did not significantly change when measured during W5 ( $20.59 \pm 0.99$  g;  $n = 8$ ), whereas at that time point, just before cannabinoid treatment, in all groups of animals repeatedly treated with

cisplatin, a reduction in the threshold for mechanical sensitivity was confirmed ( $(F_{12,114}) = 99.34$ ;  $p < 0.0001$ ; Fig. 2).

### 3.2. Effect of acute intraperitoneal administration of cannabinoids on mechanical and thermal sensitivity

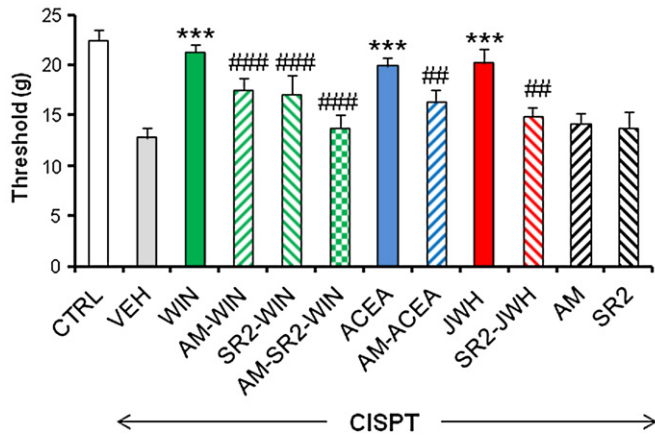
As shown in Fig. 3A, there was a significant overall group difference in mechanical sensitivity among the different groups ( $F(11,72) = 54.94$ ,  $p < 0.0001$ ). In cisplatin-treated rats, acute intraperitoneal administration of WIN reversed the threshold for mechanical allodynia to the values typical of control, saline-treated rats (Fig. 3A;  $p < 0.001$ ). This effect was sensitive to both CB1 and CB2 antagonists, and blockade was more intense when both antagonists were administered prior to WIN (Fig. 3;  $p < 0.001$ ). The selective cannabinoid drugs ACEA (CB1 agonist) and JWH133 (CB2 agonist) reversed the threshold for mechanical allodynia to values similar to that obtained in saline-treated rats (Fig. 3A;  $p < 0.001$ ). The selective cannabinoid antagonists AM251 and SR144528 respectively blocked the effect of ACEA and JWH133 (Fig. 3A;  $p < 0.01$ ). When given alone, neither antagonist altered the threshold for mechanical allodynia compared to that obtained in vehicle-injected neuropathic rats.

In the plantar test, there was a significant overall group difference in thermal sensitivity among the different groups ( $F(11,72) = 11.78$ ,

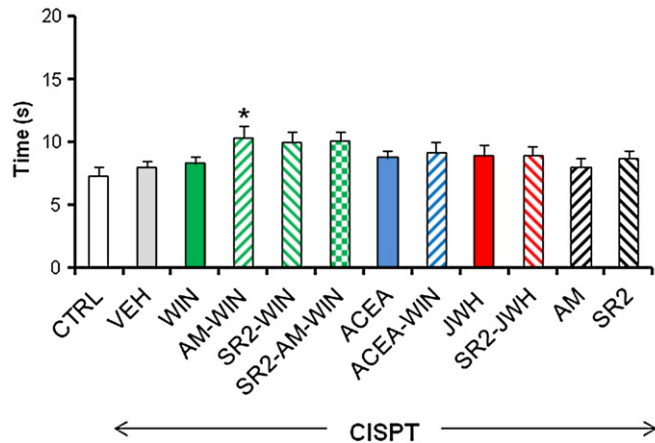


## ANTINOCICEPTIVE EFFECT OF SYSTEMICALLY ADMINISTERED CANNABINOIDS

### A) MECHANICAL SENSITIVITY



### B) THERMAL SENSITIVITY



$p < 0.0001$ ), but practically none of the treatments produced withdrawal times significantly different from cisplatin-treated rats, which had received the vehicle for cannabinoids (Fig. 3B). The only difference was that upon combination of WIN and the CB1 antagonist AM251, the withdrawal latency was slightly but significantly higher than in vehicle-treated animals (Fig. 3B).

#### 3.3. Central effects of cannabinoids intraperitoneally administered (cannabinoid tetrad)

In naïve animals, acute administration of WIN at a high concentration (5 mg/kg) induced analgesia (Fig. 4A), catalepsy (Fig. 4B) and

hypothermia (Fig. 4C). Also spontaneous locomotor activity (Fig. 4D) was decreased in comparison with vehicle-treated rats, but the corresponding values did not reach statistical significance. However, WIN, ACEA and JWH133 at 1 mg/kg did not induce any central effect in naïve animals.

In cisplatin-induced neuropathic rats, the different cannabinoid agonists (WIN, ACEA or JWH133, all at 1 mg/kg, the dose that relieved neuropathy) did not produce significant analgesia (Fig. 4A), catalepsy (Fig. 4B), hypothermia (Fig. 4C) or hypolocomotion (Fig. 4D), compared to vehicle.

#### 3.4. Effect of cannabinoids locally administered

Upon intraplantar administration, WIN increased the threshold for mechanical allodynia in the ipsilateral paw of cisplatin-treated rats (Fig. 5A). WIN effect was sensitive to the CB1 but not to the CB2 antagonist (Fig. 5A). When the CB1 and CB2 selective agonists (ACEA and JWH133, respectively) were intraplantarly administered, both of them slightly but significantly increased the threshold for mechanical allodynia in the ipsilateral paw (Fig. 5A); these effects were antagonized by the selective antagonists AM251 and SR144528, respectively. In the contralateral paw, the threshold for mechanical allodynia was slightly but significantly increased in the JWH133-treated group (Fig. 5A). When given alone, neither antagonist significantly altered the threshold for mechanical allodynia.

As for intraperitoneal administration, thermal sensitivity of cisplatin-treated rats was not significantly altered by intraplantar administration of the different cannabinoid agonists and/or antagonists (Fig. 5B).

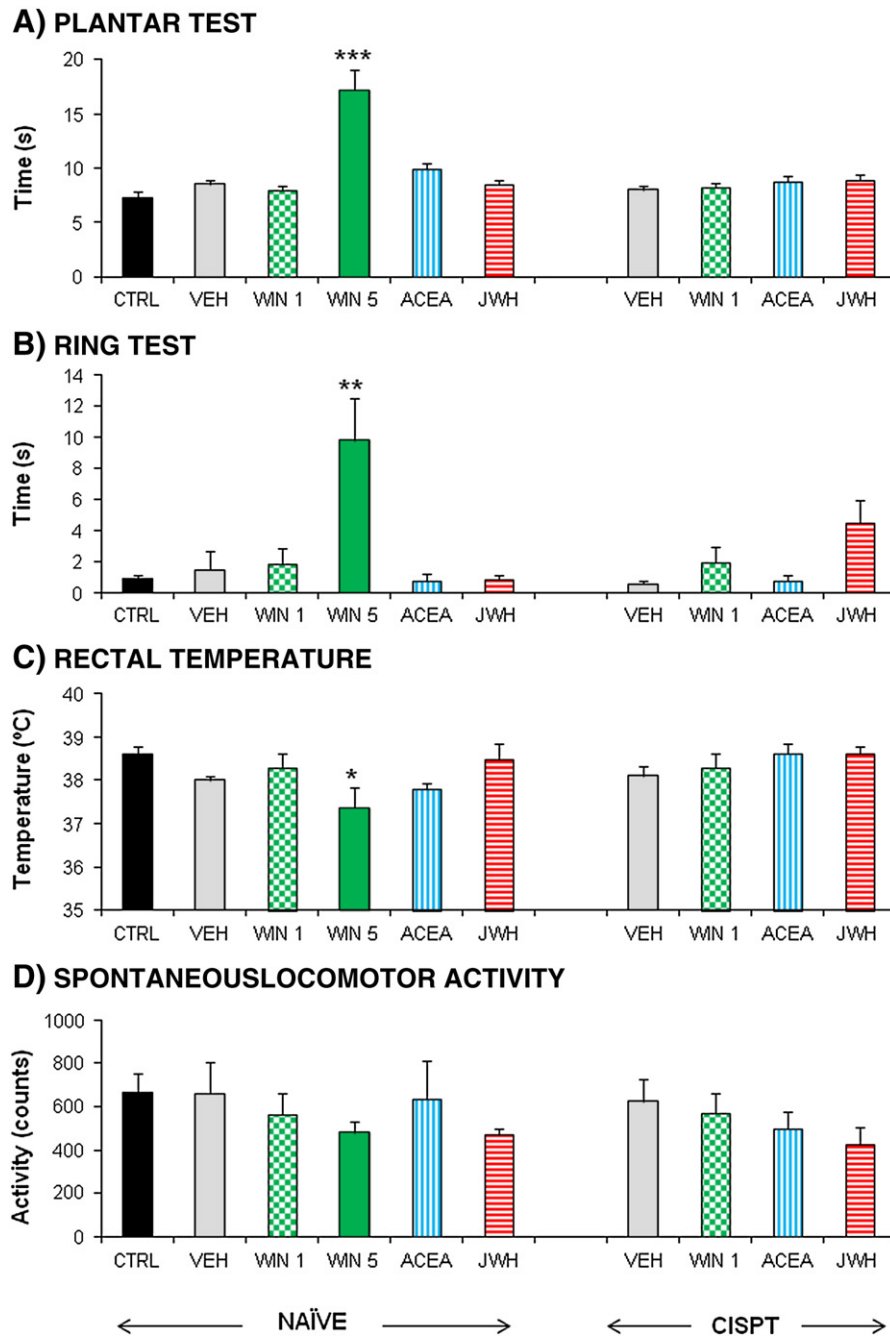
## 4. Discussion

In the present study, the mixed cannabinoid agonist WIN 55,212-2, and the selective CB1 and CB2 agonists (ACEA and JWH133, respectively), alleviated the signs of peripheral neuropathy in cisplatin-treated rats. Whereas systemic administration of cannabinoids, at a non-psychoactive dose, involved both CB1 and CB2 cannabinoid receptors, the role of CB2 receptors upon intraplantar administration of cannabinoids is less clear. Thus, different strategies implicating the activation of cannabinoid receptors may be useful to alleviate chemotherapy-induced neuropathy: the systemic administration of non-selective or CB1 selective agonists at non-psychoactive doses; the systemic administration of CB2 agonists, devoid of central effects; the local administration of cannabinoid agonists.

#### 4.1. Development of peripheral neuropathy

The use of chemotherapeutic agents in the treatment of cancer is frequently associated to the presence of painful peripheral neuropathy. Neuropathic pain symptoms associated with each chemotherapeutic agent vary and can respond differently to pharmacological treatments (Flatters and Bennett, 2004). So, distinct mechanisms may underlie the development of neuropathic pain induced by different antineoplastic agents (for review, see Cata et al., 2006) and treatment could also differ. Specifically, the neurotoxicity induced by platinum-derived drugs is characterized by a dose-dependent painful sensory neuropathy presenting with symptoms in the distal extremities of the patients (Quasthoff and Hartung, 2002).

In the present study, cisplatin treatment induced tactile mechanical allodynia but no alteration in thermal sensitivity to noxious stimuli. Our data on mechanical allodynia confirmed previous studies using a similar repeated-cisplatin administration protocol from our laboratory (Vera et al., 2011, 2007; Cabezos et al., 2010) and other's (Viana-Cardoso et al., 2011; Hori et al., 2010; Authier et al., 2003). On the contrary, the effects produced by cisplatin on thermal sensitivity remain controversial. Some studies have reported thermal hypoalgesia (Authier et al., 2003; Boyle et al., 1999) or hyperalgesia (Ta et al., 2009) whereas others



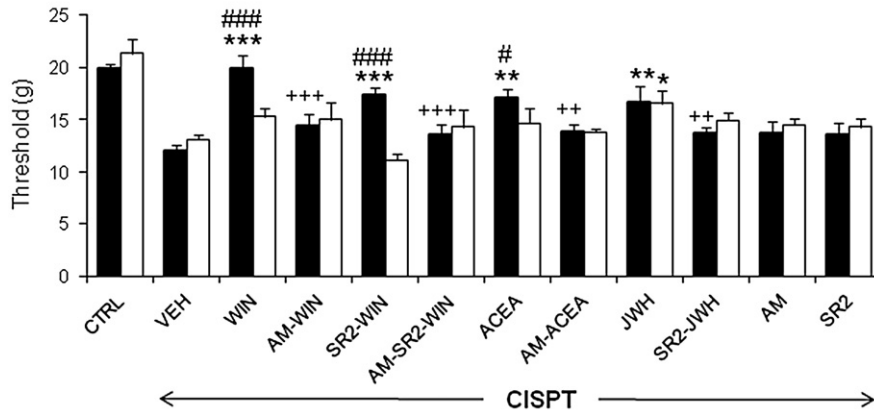
have found no change (Hori et al., 2010; De Koning et al., 1987; Tredici et al., 1999; present results) in the responses to thermal stimulation following treatment with cisplatin. In humans, the heat pain threshold did not change in cisplatin-treated patients after 3, 6 or 9 cycles of chemotherapy (Attal et al., 2009). The use of different species, strains, drug doses, and heat intensities in the above mentioned studies might explain these different results. Further work is needed to better define the alteration of thermal sensitivity in cisplatin-induced neuropathy.

#### 4.2. Effect of acute intraperitoneal administration of cannabinoids on mechanical and thermal sensitivity

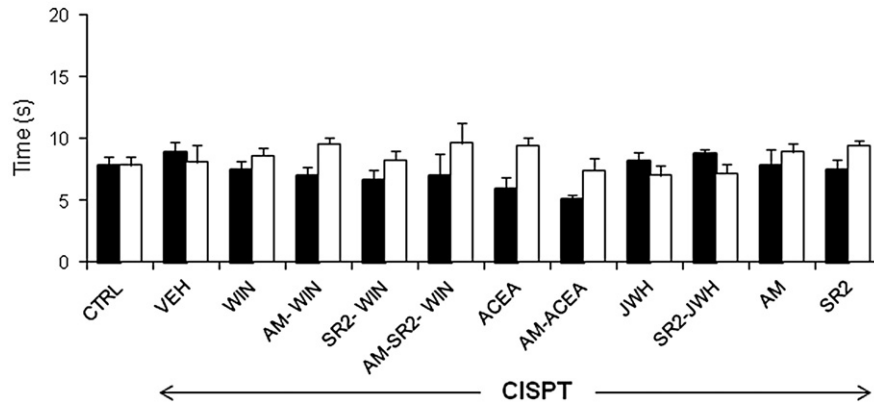
Pharmacotherapy for antineoplastic-induced neuropathy is limited because the underlying cellular mechanisms remain incompletely understood. Amytriptyline, gabapentin, opioids and many others (such as amifostine, reduced glutathione, calcium and magnesium infusions, glutamine, acetyl-L-carnitine or vitamin E) are used to treat neuropathy.

## ANTINOCICEPTIVE EFFECT OF LOCALLY ADMINISTERED CANNABINOIDS

### A) MECHANICAL SENSITIVITY



### B) THERMAL SENSITIVITY



But at present, the data are insufficient to conclude that any of the purported chemoprotective agents are effective in human patients (for review see [Albers et al., 2011](#)).

Cannabinoids have already shown acute analgesic properties in different models of neuropathic pain, such as that induced by nerve injury (for review, see [Rahn and Hohmann, 2009](#)), diabetes ([Vera et al., 2012](#)), paclitaxel ([Pascual et al., 2005](#); [Rahn et al., 2008](#)) or vincristine ([Rahn et al., 2007](#)).

CB1 receptors are enriched in the CNS and are also present in some peripheral tissues. CB2 receptors are present mainly in cells of the immune system, although not exclusively ([Van Sickle et al., 2005](#)) and their involvement in peripheral antinociception has been shown ([Quartilho et al., 2003](#); [Ibrahim et al., 2005](#)). Thus, in traumatic nerve injury, both CB1 ([Herzberg et al., 1997](#); [Fox et al., 2001](#)) and CB2 ([Ibrahim et al., 2003](#); [Beltramo et al., 2006](#)) receptors were involved in neuropathic antinociception. In diabetic neuropathy, WIN alleviated mechanical allodynia in rats; however, whereas both cannabinoid receptors were involved in streptozotocin-induced type 1 diabetes, in the Zucker

diabetic fatty rat, a genetic model of type 2 diabetes, WIN effects seemed to involve the activation of only CB1 receptors ([Vera et al., 2012](#)). In chemotherapy-induced neuropathy, WIN intraperitoneally administered suppressed neuropathic nociception induced by paclitaxel through a CB1 mechanism ([Pascual et al., 2005](#)); unfortunately, CB2 antagonists were not used in this work. In a posterior study, also CB2 selective agonists were able to attenuate paclitaxel-induced neuropathy ([Rahn et al., 2008](#)). In vincristine-treated rats, the suppression of neuropathy involved the activation of both CB1 and CB2 receptors when WIN was intraperitoneally administered ([Rahn et al., 2007](#)). Finally, here we show that systemic cannabinoid agonists (either non-selective or selective for CB1 or CB2 receptors) alleviate mechanical allodynia in cisplatin-treated rats in a CB1- and CB2-sensitive manner. Selective antagonists for CB1 and CB2 receptors alone did not fully antagonize the effects of WIN (see [Fig. 3](#)); however, when both antagonists were used together, full blockade of WIN was achieved. This further indicated that WIN effect is mediated through both CB1 and CB2 receptors in this model. Thus, with few exceptions (type 2 diabetes:

Vera et al., 2012), other's and our data suggest that both CB1 and CB2 agonists systemically applied might be useful to relieve many neuropathic pain conditions.

An additional result was that, in the present study, although cisplatin treatment induced no alteration in noxious thermal sensitivity, the administration of WIN together with the selective CB1 antagonist AM251 induced a slight but significant increase of the thermal withdrawal in cisplatin-treated animals. Furthermore, when combined with the CB2 antagonist, WIN also increased the thermal withdrawal, although in this case, the difference was not significant. Further research is needed to clarify the significance, if any, of these unexpected findings.

Whatever the case may be, CB1-acting agonists, when capable of crossing the blood brain barrier, may exert central effects that limit their clinical use. Accordingly, the administration of a high dose of WIN (5 mg/kg) induced the signs of the cannabinoid tetrad in naïve rats (present results; Abalo et al., 2011), which is particularly important in the case of catalepsy, which is sometimes used as a single sign of centrally-induced cannabinoid effects (Rahn et al., 2007). On the contrary, the dose of 1 mg/kg of WIN acutely administered was effective to relieve neuropathic pain (Pascual et al., 2005; Vera et al., 2007, 2012; Bujalska, 2008), and practically devoid of psychoactivity in control Wistar rats (present results; Vera et al., 2007). Here, the administration of ACEA (CB1 agonist) at 1 mg/kg in control rats did not induce any central effect either. Furthermore, in cisplatin-treated animals, the non-selective cannabinoid agonist WIN or the CB1-selective cannabinoid agonist tested (ACEA) did not exert any effect in the cannabinoid tetrad compared to vehicle-treated rats. So, at this antiallodynic dose, cannabinoids acting at the CB1 receptor had no psychoactive effects in our pathologic model.

In contrast, CB2-selective agonists are not associated with the psychoactive and motor effects typical of CB1 receptor activation (Rahn et al., 2008). We actually confirmed here that the CB2 selective agonist JWH133 (at 1 mg/kg) does not exert any effect in the cannabinoid tetrad in naïve or cisplatin-treated rats either. Thus, the fact that CB2 agonists are effective in relieving mechanical allodynia makes the CB2 receptor an attractive therapeutic target for the treatment of chemotherapy-induced neuropathic pain.

Therefore, our results suggest that cannabinoid agonists, irrespective of whether they are non-selective or selective for the CB1 or the CB2 receptor, could be systemically applied, at least at relatively low doses, to alleviate neuropathic pain.

## 5. Effect of cannabinoids locally administered

A further interesting alternative to avoid the central effects of cannabinoids is the use of the topical route of administration. When cannabinoids were locally applied, the mixed CB1/CB2 agonist WIN and the selective agonists for CB1 and CB2 receptors (ACEA and JWH133, respectively) alleviated mechanical allodynia in cisplatin-treated rats. Interestingly, however, WIN effect was only mediated by the CB1 receptor, and thus the implication of the CB2 receptor is not completely clear.

Topical application of cannabinoids has reduced pain in a human experimental model (Rukwied et al., 2003). In agreement with this, local administration of WIN has been reported to produce anti-nociceptive effects in both inflammatory (Nackley et al., 2003) and different neuropathic animal models (Fox et al., 2001; Ulugol et al., 2004; Vera et al., 2012), suggesting that the activation of peripheral cannabinoid receptors can reduce nociception independently of their effects on the CNS (Hohmann, 2002; Walker and Huang, 2002).

However in animal models of chemotherapy-induced neuropathy results are somehow controversial. Thus, in animals treated with vincristine (Rahn et al., 2007) or paclitaxel (Pascual et al., 2005) the intraplantar administration of WIN failed to suppress mechanical allodynia, whereas in a model of cisplatin-induced neuropathy in mice,

intraplantar injection of anandamide (an endogenous cannabinoid agonist capable of activating CB1 and TRPV1 receptors) attenuated thermal and mechanical hyperalgesia. In this model, the effect was mediated by CB1 but not CB2 receptors (Khasabova et al., 2012). Similarly, in the present study WIN relieved neuropathy via CB1 but not CB2 receptors when topically applied. Furthermore, ACEA was also capable of producing antinociception by this route of administration. Interestingly, local administration of WIN (and to a slightly, but significant, lower extent, ACEA) was effective to significantly reduce mechanical allodynia in the ipsilateral paw, without modifying the threshold in the contralateral paw in cisplatin-treated rats, suggesting that cannabinoids did not need to reach the CNS to exert an antiallodynic effect at the dose tested. The lack of effect of either WIN or ACEA at 1 mg/kg on the cannabinoid tetrad further suggests that their effect upon intraplantar administration may be due to activation of local CB1 receptors.

In spite of the fact that WIN local effect was not sensitive to the CB2 antagonist, JWH133 (CB2 selective agonist) was capable of reducing mechanical allodynia when topically administered. In another animal model, the chronic constriction of sciatic nerve, topical application of another CB2 selective agonist (JWH015) reduced mechanical allodynia in mice and an enhanced transcription of CB2 receptor was demonstrated in the spinal cord and dorsal root ganglia of mice after chronic constriction of sciatic nerve (Hervera et al., 2010). Similar mechanisms might be involved in our model.

It is not clear why the threshold for mechanical allodynia was modified in the contralateral paw by JWH133 topical application. In paclitaxel-induced neuropathy (Pascual et al., 2005), small doses of WIN (50 and 100 µg) were ineffective but when the effective dose (250 µg) was reached, similar results were obtained in the paw where WIN was injected and in the non-injected paw. Leakage of the cannabinoid into the systemic circulation may contribute to changes in paw withdrawal thresholds observed in the non-injected paw.

Finally, the acute administration of the CB1 and CB2 antagonists alone had no effect in cisplatin-treated rats. This suggests that endocannabinoids might not be tonically released in this model of cisplatin-induced neuropathy. In this sense, in cisplatin-treated mice, cisplatin-induced mechanical and heat hyperalgesia were accompanied by a decrease in the level of anandamide in the plantar paw skin (Khasabova et al., 2012). It was postulated that the CB1 receptor tone in nociceptors modulates the threshold for their activation (Agarwal et al., 2007); so, the reduction in the anandamide content of plantar skin in cisplatin-treated mice might contribute to the behavioral hypersensitivity observed in mice (Khasabova et al., 2012). More research is needed to determine if anandamide is also decreased in our model. However, it is also likely that a floor effect might be present where responses to punctate mechanical stimuli will not decrease below those observed.

## 6. Conclusion

In cisplatin-induced neuropathy, cannabinoid agonists produce an antinociceptive effect when locally or systemically applied. Whereas the participation of CB1 receptors is demonstrated for both routes of administration, that of CB2 receptors at the local level is not so clear. Although low doses of cannabinoid agonists (even those acting at CB1 receptors and capable of crossing the blood brain barrier), might be useful to relieve neuropathic pain, our results suggest that local administration of selective CB1 agonists or systemic administration of CB2 agonists may serve as new therapeutic alternatives for symptom management in painful neuropathy associated with cisplatin treatment.

## Competing interests

The authors declare that they have no competing interests.

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