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

Gema Vera *, Pablo Antonio Cabezos, María Isabel Martín, Raquel Abalo

Departamento de Farmacología y Nutrición, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos., Avda. de Atenas s/n., 28922 Alcorcón, Madrid, Spain

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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.

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.

* Corresponding author. Tel.: +34 91 488 90 86; fax: +34 91 488 89 55.
E-mail address: gema.vera@urjc.es (G. Vera).

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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