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Original Article

Preventive and therapeutic effects of JM-20 on paclitaxel-evoked painful peripheral neuropathy in rats

[Efectos preventivo y terapéutico del JM-20 sobre la neuropatía periférica dolorosa inducida por paclitaxel en ratas]

Bárbara B. Garrido-Suárez[1,2*], Gabino Garrido[3], Addis Bellma Menéndez[1], Guillermo Aparicio[1], Odalys Valdés[1], Estael Ochoa-Rodríguez[4], Yamila Verdecia-Reyes[4], René Delgado-Hernández[1]

[1]Laboratorio de Farmacología y Toxicología, Centro de Investigación y Desarrollo de Medicamentos. Ave. 26 No. 1605 e/ Boyeros y Puentes Grandes, Nuevo Vedado, Plaza. Apdo. Postal 10600. La Habana, Cuba.
[2]Departamento de Farmacología, Instituto de Ciencias del Mar. Calle Loma entre 35 y 37, Alturas del Vedado, Plaza de la Revolución, Apdo. Postal. 10400. La Habana, Cuba.
[3]Departamento de Ciencias Farmacéuticas, Facultad de Ciencias, Edificio Ñ3, Universidad Católica del Norte, Angamos 0610, Antofagasta, Chile.
[4]Laboratorio de Síntesis Orgánica de La Facultad de Química de La Universidad de La Habana, Zapata s/n entre G y Carlitos Aguirre, Vedado, Plaza de la Revolución, CP 10400, La Habana, Cuba.
*E-mail: beatriz.garrido@infomed.sld.cu

Abstract

Context: JM-20 is a hybrid synthetic molecule, which is based on a multimodal drug design paradigm for cerebrovascular disease. In addition to its neuroprotective effects, JM-20 also decreased sciatic nerve chronic constriction injury (CCI)-induced mechanical hypersensitivity in rats. JM-20 has a strong mitoprotective ability, and its effects could be in correspondence with the mitotoxicity hypothesis for paclitaxel-induced painful peripheral neuropathy.

Aims: To evaluate the efficacy of the JM-20 to reduce neuropathic pain manifestations induced by the administration of paclitaxel in rats.

Methods: In this study was implemented a rat model of painful peripheral neuropathy, produced by the chemotherapeutic agent paclitaxel, to determine whether JM-20 (10 mg/kg, p.o) could prevent the development of neuropathic pain during the exposure to paclitaxel. As well as to determine whether JM-20 (20 mg/kg, p.o) could reverse the established neuropathic pain. Mechanical behavioral assessment using von Frey filaments applied to the hind paws was applied before, during, and after treatments for 35 days.

Results: Giving JM-20 during the exposure to paclitaxel significantly reduced the severity of mechanical allodynia and mechanical hyperalgesia. Moreover, JM-20 significantly reduced both established neuropathic pain manifestations. There was no evidence of tolerance to the effect during three days of dosing, and a long-term effect was observed after JM-20 discontinuation.

Conclusions: JM-20 may be clinically relevant for both the prevention and treatment of paclitaxel-induced painful peripheral neuropathy.

Keywords: chemotherapy; JM-20; mitochondria; neuropathic pain; neuroprotection.

Resumen

Contexto: El JM-20 es una molécula sintética hibrida, la cual está basada en un paradigma de diseño de fármacos multimodales para la enfermedad cerebrovascular. Además de sus efectos neuroprotectores, JM-20 también disminuye la hipersensibilidad mecánica inducida por la constricción crónica del nervio ciático en ratas. JM-20 posee habilidad mitoprotectora potente y sus efectos podrían estar en correspondencia con la hipótesis de la mitotoxicidad para la neuropatía periférica dolorosa inducida por paclitaxel.

Objetivos: Evaluar la eficacia de JM-20 para reducir las manifestaciones de dolor neuropático inducidas por la administración de paclitaxel en ratas.

Métodos: En este estudio se implementó el modelo de neuropatía periférica dolorosa producido por el agente quimioterapéutico, paclitaxel, para determinar si JM-20 (10 mg/kg, p.o.) podría prevenir el desarrollo del dolor neuropático durante la exposición al paclitaxel. Así como para determinar si JM-20 (20 mg/kg, p.o.) podría revertir el dolor neuropático establecido. Se ejecutó la evaluación conductual ante los estímulos mecánicos mediante la aplicación de los filamentos von Frey en las patas traseras antes y después de los tratamientos por 35 días.

Resultados: La administración de JM-20 durante la exposición al paclitaxel redujo significativamente la severidad de la alodinia mecánica y la hiperalgesia mecánica. Además, JM-20 redujo significativamente ambas manifestaciones de dolor neuropático establecidas. No hubo evidencias de tolerancia al efecto durante los 3 días de dosificación y se observó el efecto a largo plazo del JM-20 tras su discontinuación.

Conclusiones: JM-20 puede tener relevancia clínica para la prevención y tratamiento de la neuropatía periférica dolorosa inducida por paclitaxel.

Palabras Clave: dolor neuropático; JM-20; mitocondria; neuroprotección; quimioterapia.

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AUTHOR INFO
ORCID: 0000-0002-4547-4109 (GG)
0000-0002-0541-1816 (EOR)
0000-0001-7051-7871 (RDH)

INTRODUCTION

Cancer chemotherapy-induced peripheral neuropathy (CIPN) is the major dose-limiting toxicity associated with cancer treatment, which can lead to a suboptimal treatment or its discontinuation with consequences for prognosis (Sisignano et al., 2014). Frequently neuropathic pain (NP) persists long after completion of chemotherapy, thereby reducing cancer survivors' quality of life (Seretny et al., 2014). The incidence of CIPN varies between 3–7% using a single agent but can rise to 38% with combination regimens (Bennett et al., 2012; Jaggi and Singh 2012). CIPN is a severe adverse effect of several cytostatic drugs (taxanes, platinum derivatives, vinca alkaloids, epothilones, bortezomib, and thalidomide) (Sisignano et al., 2014; Ventzel et al., 2016). It evokes a range of symptoms such as numbness and tingling, mechanical allodynia, cold allodynia, and on-going burning pain. Unfortunately, the pathophysiological mechanisms are not fully elucidated (Boyette-Davis et al., 2015). Furthermore, there are no available therapies to prevent or minimize CIPN, and only few pharmacological strategies exist for its treatment (Flatters and Bennett, 2004; Gewandter et al., 2017). To face this problem, neuroprotective strategies in a mechanism-based manner have been recommended to evaluate in the clinical setting (Sisignano et al., 2014; Krukowski et al., 2015). Currently, mitochondrial dysfunction has been proposed to be a relevant mechanism for chemotherapy-induced neuropathy (Flatters and Bennett, 2006; Flatters et al., 2006; Siau et al., 2006; Xiao et al., 2009). The opening of mitochondrial permeability transition pore (mPTP) may release cytochrome C to initiate apoptotic cascade with activation of calpains/caspases, which induce neuronal cytotoxicity. Hence, there may be a loss of Aδ and C fibers from the epidermis, including nociceptors in the form of loss of intra-epidermal nerve fibers. The transected nerve fibers/degenerated terminal arbors acquire spontaneous discharge and mechanical sensitivity due to hyper-responsiveness of remnant nociceptors (Kidd et al., 2002; Cata et al., 2006; Siau et al., 2006; Jin et al., 2008; Xia and Bennett, 2008; 2012).

JM-20 is a hybrid molecule composed of 1,5-benzodiazepine (BDZ) fused to a dihydropyridine (DHP) moiety. It is based on a multimodal drug design paradigm for cerebrovascular disease (Nuñez-Figueredo et al., 2013). This molecule possesses GABAergic activity and a robust neuroprotective ability, as demonstrated in models relevant to cerebral ischemia and related to anti-excitotoxic, anti-inflammatory, anti-apoptotic, mitoprotective, and antioxidant effects (Nuñez-Figueredo et al., 2013; 2014a; 2014b; 2014c; 2015; Ramírez-Sánchez et al., 2015; 2018). Recently, JM-20 decreases chronic constriction injury (CCI)-induced mechanical hypersensitivity in relation to its preventive effect on Wallerian degeneration (WD), has been reported (Garrido-Suárez et al., 2020). All these pharmacological effects make this molecule attractive to be explored in the context of CIPN. Particularly, JM-20 prevents Ca^{2+}-induced mitochondrial permeability transition in rat brain mitochondria (Nuñez-Figueredo et al., 2014b). Afterward, according to the mitotoxicity hypothesis, JM-20 has the potential to succeed in mitochondrion-targeted strategies to oppose chemotherapy-induced painful peripheral neuropathy.

Here, we used a rat model of painful peripheral neuropathy produced by the chemotherapeutic agent, paclitaxel, to determine whether JM-20 could prevent the development of neuropathic pain syndrome during the exposure to paclitaxel. In the same way to determine whether JM-20 could reverse established neuropathic pain.

MATERIAL AND METHODS

Experimental animals

Experimental procedures were carried out in accordance with European regulations on animal protection (Directive 86/609), the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (NIH publication 85-23, revised 1996). All experimental protocols were approved by the Institutional Animal Care and Ethical Committee from the Center of

Drugs Research and Development (CIDEM, La Habana, Cuba). Male Sprague-Dawley (8-10 weeks) rats weighing 200-250 g were obtained from the Center for Experimental Animals Production (CENPALAB, La Habana, Cuba). They were kept in controlled conditions (22 ± 0.5°C, relative humidity 40-60%, a 12-hour light/dark cycle [light on from 07:00 to 19:00 h], and food and water available *ad libitum*). The experiments took place during the light period, and animals in each treatment group (n = 8-10 for each group) were tested in a randomized order.

Drug administration

Paclitaxel, 2 mg/kg per mL, was prepared fresh daily by diluting generic paclitaxel developed in the CIDEM Cytostatics Plant similar to paclitaxel (Taxol; Bristol-Myers-Squibb; 6 mg/mL in a 50:50 mixture of ethanol and Cremofor). This was diluted with saline to a concentration of 2 mg/mL and injected intraperitoneally (i.p.) on 4 alternate days (day 0 [D0], D2, D4, and D6) (Polomano et al., 2001; Flatters and Bennett, 2004). Control healthy animals received injections of similar volume (1.0 mL/kg) of the vehicle. This was prepared from the stock solution Cremofor and dehydrated ethanol 95% in a 1: 1 ratio diluted in two parts of the saline solution. JM-20 was synthesized, purified, and characterized as previously reported (Nuñez-Figueredo et al., 2013). The compound was supplied by the Laboratory of Organic Synthesis from the Faculty of Chemistry of Havana University (Cuba). Immediately before use, JM-20 was suspended in 0.05% carboxymethyl cellulose (CMC) for oral administration.

Behavioral testing

All animals were habituated to the behavioral testing environment, and three baseline measurements of mechanical sensitivity were taken prior to paclitaxel or vehicle administration. For each testing session, animals were placed in Plexiglas cages (21 × 26 × 27 cm) with a wire grid bottom and allowed to acclimatize for 10 min. Mechanical allodynia and mechanical hyperalgesia were assessed using three von Frey filaments (Stoelting, Wood Dale, IL, USA) with bending forces of 4, 8, and 15 g. In ascending order of force, each filament was applied to the mid-plantar area (avoiding the base of the tori) of each hind paw 5 times, with each application held for 5 s. Withdrawal responses to the von Frey filaments from both hind paws were counted and then expressed as an overall percentage response. Normal rats rarely withdraw from the 4 g stimulus; the increased level of responding is thus indicative of mechanical allodynia. Normal animals withdraw from the 15 g stimulus 15-20% of the time, the increased level of responding seen after paclitaxel treatment is thus indicative of mechanical hyperalgesia, and responses to 8 g are intermediate (Flatters and Bennett, 2004).

Experimental protocols

Preventive parading

In order to discern whether this molecule could prevent painful peripheral neuropathy induced by paclitaxel, a preventive design was carried out. The animals were divided into 3 groups (each n = 8): neuropathic group treated with paclitaxel and CMC (0.05%) vehicle (10 mL/kg, p.o.), naive group treated with vehicle i.p. and CMC (0.05%) vehicle and experimental JM-20 neuropathic group treated with paclitaxel and JM-20 (10 mg/kg, p.o.). Oral treatment with JM-20 or vehicle starting the day prior to the first injection of paclitaxel (D1) during consecutive 17 days, and the last dose of JM-20 was 9 days after the last dose (D6) of taxol. On those days when both drugs were to be administered, JM-20 was given at 8:00 h and paclitaxel at 13:00 h. The measurements were carried out on D20, D25, D27, D29, and D35 post-taxol in correspondence with the maximum clinical expression of neuropathic manifestations (between 23-24 to 28 days) (Polomano et al., 2001; Flatters et al., 2006; Xiao et al., 2009).

Therapeutic parading

To determine whether JM-20 has an anti-hyperalgesic effect on established paclitaxel-evoked pain, a therapeutic design was also carried out during the period of approximate peak pain severity. The animals were allocated in three

groups (each n = 8-10) were then randomly assigned to receive JM-20 (20 mg/kg) or vehicle on three consecutive days, beginning on D27 (Xiao et al., 2009). On the first single dose of JM-20, the withdrawal responses were evaluated before, 1, and 3 h after treatment. Behavior was examined daily 1 h after the treatment on D28 and D29. In addition, the animals were evaluated at 35 days post-taxol (D35, 6 days after the third dose) after a washout period to evaluate possible long-term effects of JM-20.

Histopathological study

For histopathological examination, biopsies of paw skin of the rats were taken on the D35 after the induction of CIPN. The tissue slices were fixed in 10% neutral buffered formaldehyde for 5 days, embedded in paraffin, and sectioned into 4 µm thickness using an Olympus microtome. Staining was carried out by using hematoxylin and eosin (HE) and were analyzed qualitatively under light microscope (40×).

Statistical analysis

Data were analyzed using the statistical program Graph Pad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Inter-group statistically significant differences were tested using a one-way analysis of variance (ANOVA) followed by Bonferroni's or Dunnett's *posthoc* tests for multiple comparisons. The results are presented as mean ± SEM. $P<0.05$ was considered statistically significant.

RESULTS

Preventive effects of JM-20 on paclitaxel-induced painful peripheral neuropathy

Paclitaxel-treated rats that received a vehicle showed a marked, prolonged mechanical allodynia/hyperalgesia evident at day 20 (plateau D25-D29) and persisted until day 35 post paclitaxel initiation. The animals pretreated with JM-20 showed a significant reduction in the percentage of withdrawal response with the 4 g, 8 g, and 15 g filaments from D20 until the end of the study on D29-35 compared with control animals treated with vehicle. In Fig. 1A-B, the effect of JM-20 on the percentage of response to the 4 g and 15 g filaments is shown. The analysis of the area under the curve (AUC) D0-D35 is inserted to the right.

Therapeutic effect of JM-20 on paclitaxel-induced painful peripheral neuropathy

Likewise, a single dose of JM-20 reversal of established mechanical allodynia and hyperalgesia at day 27 post paclitaxel initiation, the therapeutic effect was observed at 1 h and remained at 3 h after a single dose of JM-20 (Fig. 2A-B). Treatment paradigm showing the effects of three daily doses of JM-20 on established paclitaxel-evoked neuropathic manifestations. JM-20 significantly suppressed both sensory disturbances after the 1st administration and continued to produce the same effect after the 2nd–3rd administrations during D27, D28, and D29 post-paclitaxel, respectively. Then, there was no indication of the development of tolerance to the anti-allodynic and anti-hyperalgesic effects. In addition, 6 days after JM-20 discontinuation, the anti-allodynic effect persisted on D35. The inhibition of the overall percentage response to the 15 g stimulus was not statistically significant. Possibly this change was not evident because, at D35, hyperalgesia was declined in polyneuropathic animals (Fig. 3A-B).

Histopathological analysis of skin from the paws

The histopathological analysis of the paw skin of rats injected with paclitaxel showed the presence of little lacunar spaces between collagen fibers, suggesting discrete edema of conjunctive tissue, without any other sign of inflammation compared with naive animals (Fig. 4A-B). The animals pretreated with JM-20 showed near-normal morphology, suggesting that JM-20 prevented the development of peripheral edema induced by this chemotherapeutic agent.

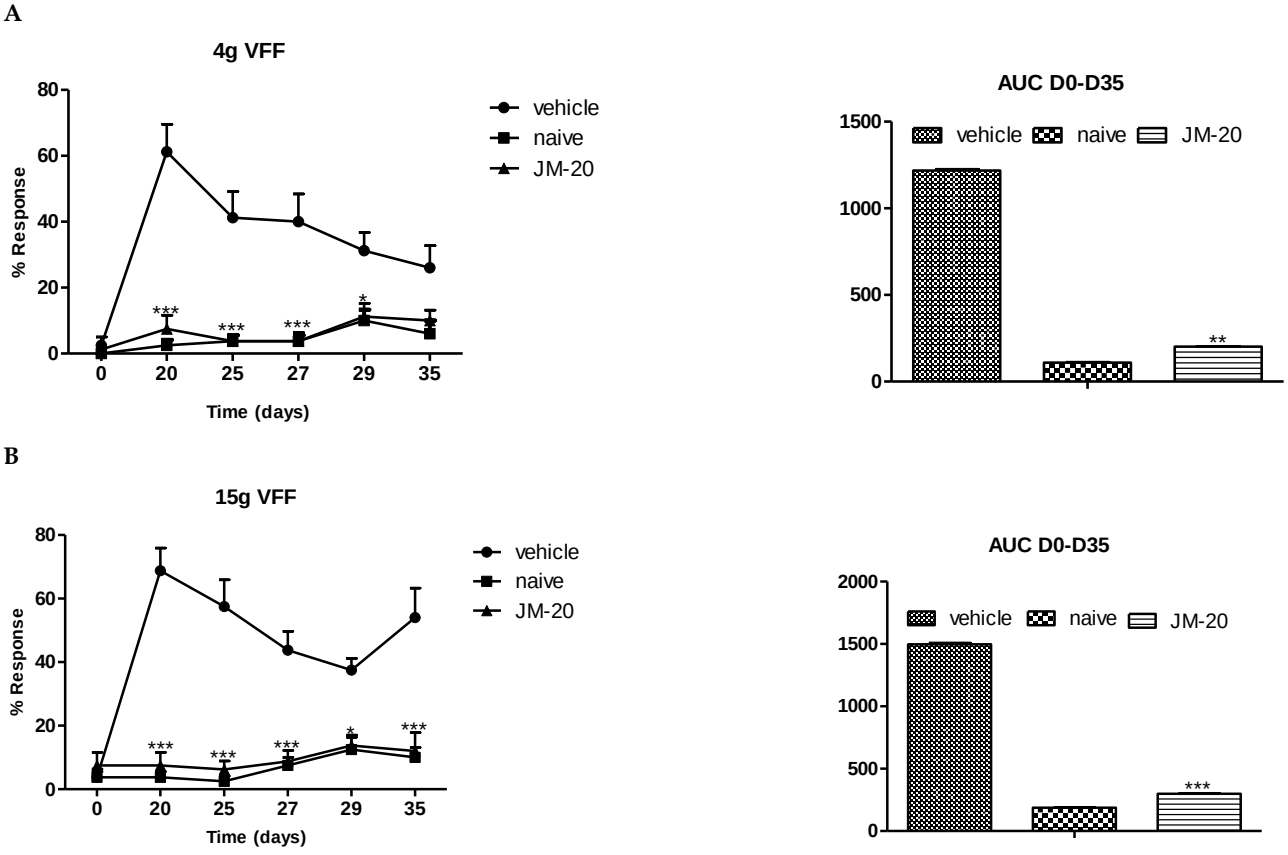

Figure 1. Preventive paradigm.

Prophylactic treatment with JM-20 (10 mg/kg, p.o.) significantly reduces the development of paclitaxel-evoked mechanical allodynia (mean ± SEM response frequency to 4 g von Frey hair, **A**) and mechanical hyperalgesia (15 g von Frey hair, **B**) in rats. JM-20 or vehicle (CMC 0.05%) were administered daily for 17 days, beginning the day before the first injection of paclitaxel, and continuing for 9 days after the last paclitaxel dose. The lateral panel shows area under the curve (AUC) analyses for D0-D35 (the ordinates show arbitrary units). The data represent the mean ± SEM (n = 8). One-way analysis of variance (ANOVA) followed by Dunnett post hoc test, $^{*}p<0.05$ and $^{***}p<0.001$ *vs.* vehicle.

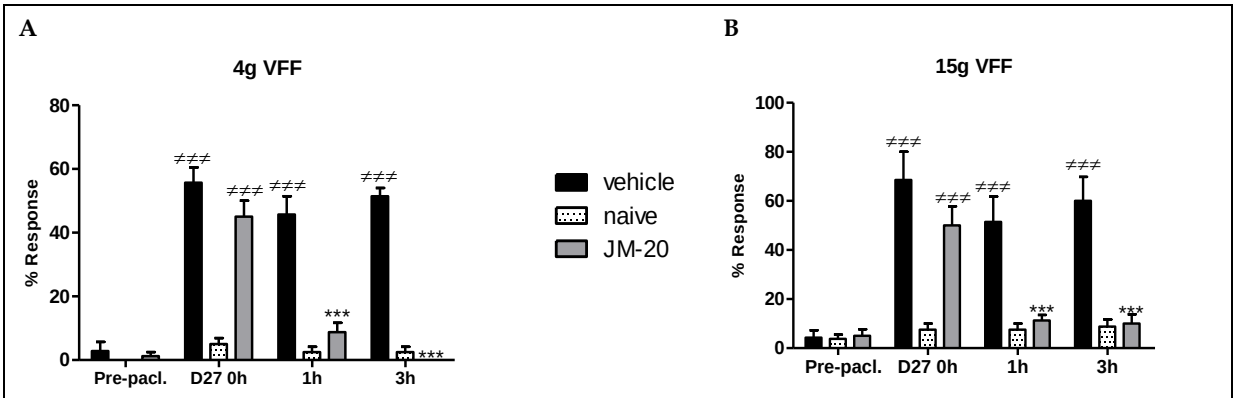

Figure 2. Treatment paradigm showing the effects of a single dose of JM-20 (20 mg/kg, p.o.) on established paclitaxel-evoked neuropathic: **(A)** mechanical allodynia (4 g VFF) and **(B)** mechanical hyperalgesia (15 g VFF).

Pre-dosing: Paclitaxel-treated rats show statistically significant mechanical allodynia and mechanical hyperalgesia on D27 after paclitaxel administration compared with control naive animals. The therapeutic effect at 1 h after a single dose of JM-20 persisted until 3 h of observation. The data represent the mean ± SEM (n = 8-10). One-way analysis of variance followed by Bonferroni post hoc test was performed, $^{***}p<0.001$ *vs.* vehicle, $^{\#\#\#}p<0.001$ *vs.* naive).

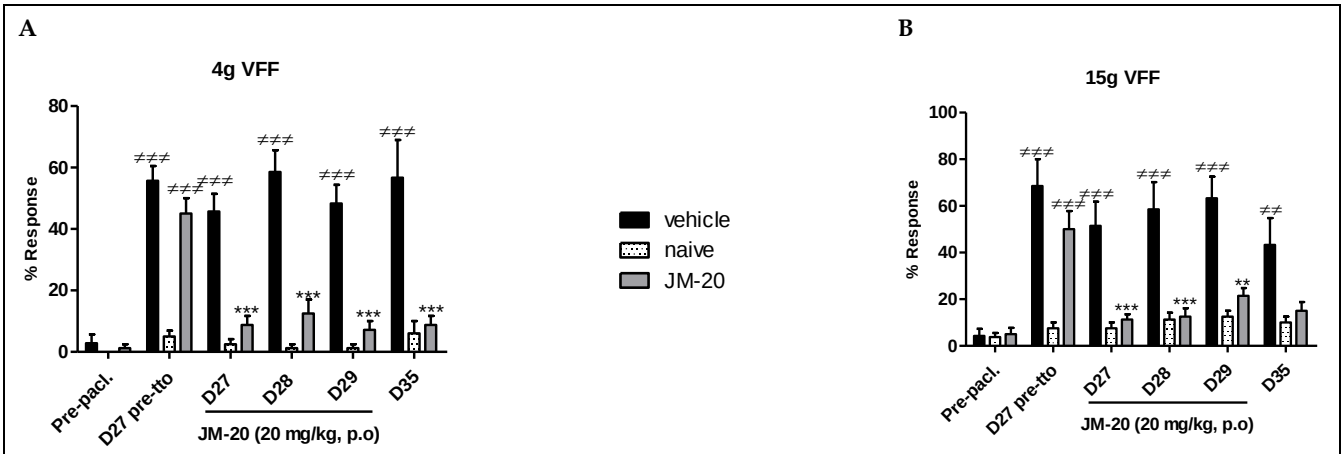

Figure 3. Treatment paradigm showing the effects of a single dose of JM-20 (20 mg/kg, p.o.) on established paclitaxel-evoked neuropathic: **(A)** mechanical allodynia (4-g VFF) and **(B)** mechanical hyperalgesia (15-g VFF).

Pre-dosing: Paclitaxel-treated rats show statistically significant mechanical allodynia and mechanical hyperalgesia on D27 after paclitaxel administration compared with control naive animals. Daily dosing: effects of the 1st-3rd administrations of JM-20. Washout days: persistent anti-allodynia on D35 after JM-20 discontinuation. The data represent the mean ± SEM (n = 8-10). One-way analysis of variance followed by Bonferroni post hoc test was carry-out, $^{**}p<0.01$ and $^{***}p<0.001$ *vs.* vehicle, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ *vs.* naive.

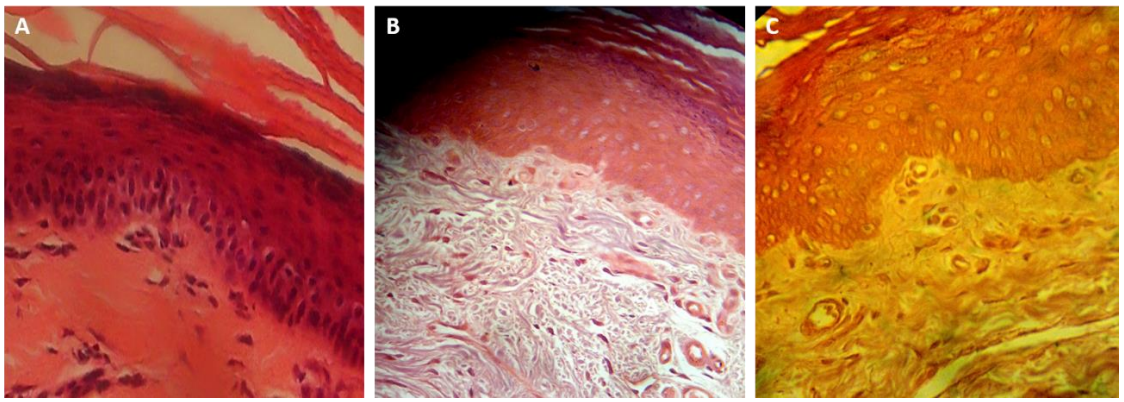

Figure 4. Photomicrographs of the skin from the hindpaw of rats on D35 after the experimental peripheral neuropathy due to systemic administration of paclitaxel (HE staining, 40×) in rats.

(A) Naive rats, the image shows the normal appearance of dermis and subdermis; **(B)** Control rats treated with vehicle (CMC 0.05%, 10 mL/kg, p.o.) shows lacunar spaces between collagen fibers, suggesting discrete edema of conjunctive tissue; **(C)** Rats pre-treated with JM-20 (10 mg/kg, p.o.) in the context of the preventive paradigm shows near-normal morphology, suggesting that JM-20 prevent the development of edema.

DISCUSSION

JM-20 given prophylactically significantly reduces paclitaxel-evoked mechanical hypersensitivity. This molecule was also effective in decreases established neuropathic manifestations in the CIPN model, and there was no evidence of tolerance to its effect. Paclitaxel-induced-allodynia and hyperalgesia are associated with the appearance of atypical axonal mitochondria (swollen and vacuolated) (Flatters and Bennett, 2006). Then, mitochondrial dysfunction of sensory nerves and consequent energy failure has been proposed to cause axonal degeneration and pain (Flatters et al., 2006; Jin et al., 2008; Xia and Bennett, 2012). JM-20 elicits neuroprotective action through a pleiotropic mechanism, which included its mitoprotective effects against Ca^{2+}-induced impairment in addition to ATP preservation (Nuñez-Figueredo et al., 2014a; 2014b). The administration of drugs such as acetyl-

L-carnitine, alpha-lipoid acid, and olesoxime (cholest-4-en-3-one, oxime), which are known to have mitotoprotective effects, has been effective in vincristine-and paclitaxel-induced painful neuropathy in animals (Xia and Bennett, 2008; Bordet and Pruss, 2009; Xiao et al., 2009). The long-term treatment effects of mitoprotective agents have been related to the prevention of axonal degeneration, which in this context is confined to the region of the sensory fiber's receptor terminals in the skin and associated with activation of cutaneous Langerhans cells (LC) (Siau et al., 2006; Wolf et al., 2008). However, the effect of JM-20 on the loss of intra-epidermal nerve fibers and LCs in this model should be elucidated. JM-20 protects against sciatic nerve myelin degradation and loss in the CCI model (Garrido-Suárez et al., 2020). Mitochondria might be an important node in the regulation and execution of WD. Before axon fragmentation, a loss of mitochondrial potential associated with mitochondrial swelling is observed (Summers et al., 2014). Interestingly, pharmacological drugs that inhibit the opening of the mitochondrial permeability transition pore extend axon survival in transected sciatic nerve (Barrientos et al., 2011). In particular, the pivotal role of Schwann cell mitochondria during Wallerian demyelination has been recognized (Tricaud and Park, 2017). JM-20 prevents the Ca^{2+}-induced mitochondrial permeability transition, as assessed by mitochondrial swelling, potential membrane dissipation, and organelle release of the pro-apoptotic protein cytochrome c in rat liver and brain mitochondria (Nuñez-Figueredo et al., 2014b). Nevertheless, since neuropathy induced by paclitaxel involves a strong peripheral and central inflammatory component, the ability of JM-20 to decrease the peripheral neuroinflammatory reaction, as well as to inhibit glial activation and apoptotic cell signaling pathways, could also involve in its preventive effect (Boyette-Davis et al., 2015; Ramírez-Sánchez et al., 2015; 2018; Garrido-Suárez et al., 2020). JM-20 can decrease plasma extravasation and tumor necrosis factor-alpha production in the inflammatory model (Garrido-Suárez et al., 2020). Paclitaxel-induced vascular hyperpermeability has been closely associated with sensory neuropeptides, which also shows alterations in paclitaxel-induced painful peripheral neuropathy (Itoh et al., 2004; Ko et al., 2014; Pittman et al., 2014). Here we also observed that JM-20 prevents the development of peripheral cutaneous edema, a known adverse effect of taxane chemotherapy (Brønstad et al., 2004; Sibaud et al., 2016).

On the other hand, neuroprotective agents might have short-term effects by improving metabolic imbalance in sensory neurons, in turn, its ability to operate ion transporters and ameliorating acute allodynia and hyperalgesia (Xia and Bennett, 2008). Here, JM-20 also decreases established NP manifestations; accordingly, the transient activity of its BDZ portion on nociceptive pathways mediated by GABA/BDZ receptors could explicate the therapeutic effect of a single dose; however, JM-20 by maintaining the cellular energy balance, could also decrease spontaneous neural activity in these conditions. Some of the current mechanism-based recommendations for the treatment of CIPN include balancing neurotransmitter levels, decreasing excitatory synaptic activity, and reducing spinal inflammation (Sisignano et al., 2014). In particular, the reduction of spinal cord GABAergic inhibition is a major contributor to persistent NP. Thus, we hypothesize that the decrease of glutamatergic signaling by JM-20, together with its GABAergic-like effect, could restore the spinal inhibitory-excitatory tone balance in synaptic transmission. Recently, the antihyperalgesic effect of transplant-mediated enhancement of GABAergic tone in this model has been reported (Bráz et al., 2015). In addition, the long-term therapeutic effect of this compound suggests its ability to modulate synaptic plasticity. Considering that glutamatergic neurotransmission plays a pivotal role in central sensitization that occurs in NP, the regulatory effect of JM-20 on the glutamate homeostasis could be involved in this phenomenon (Latremoliere and Woolf, 2009; Nuñez-Figueredo et al., 2015). In line with these ideas, the downregulation of glutamate transporters in the spinal cord to the paclitaxel-induced hyperalgesia has been reported (Weng et al., 2005). Moreover, its DHP portion, which may confer L-type voltage-gated calcium channel (VGCC) block-

ing properties, could prevent long-lasting adaptations regulating somatic calcium signals involved in transcription-dependent synaptic plasticity (Wiegert and Bading, 2011).

CONCLUSIONS

Although the present results show the preventive and therapeutic effects of JM-20 on paclitaxel-evoked painful peripheral neuropathy in rats, the underlying mechanism for its anti-neuropathic mechanisms has recently begun to be elucidated. Continued research into the mechanisms through which JM-20 can prevent and reduce mechanical hypersensitivity according to the mitotoxicity hypothesis and other targets will help develop effective interventions for CIPN.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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REFERENCES

Barrientos SA, Martinez NY, Yoo S, Jara JS, Zamorano S, Hetz C, Twiss JL, Alvarez J, Court FA (2011) Axonal degeneration is mediated by the mitochondrial permeability transition pore. J Neurosci 31: 966-978.

Bennett MI, Rayment C, Hjermstad M, Aass N, Caraceni A, Kaasa S (2012) Prevalence and aetiology of neuropathic pain in cancer patients: a systematic review. Pain 153: 359–365.

Bordet T, Pruss RM (2009) Targeting neuroprotection as an alternative approach to preventing and treating neuropathic pain. Neurotherapeutics 6: 648–662.

Boyette-Davis JA, Walters ET, Dougherty PM (2015) Mechanisms involved in the development of chemotherapy-induced neuropathy. Pain Manag 5: 285–296.

Bráz JM, Wang X, Guan Z, Rubenstein JL, Basbaum AI (2015) Transplant-mediated enhancement of spinal cord GABAergic inhibition reverses paclitaxel-induced mechanical and heat hypersensitivity. Pain 156: 1084–1091.

Brønstad A, Berg A, Reed RK (2004) Effects of the taxanes paclitaxel and docetaxel on edema formation and interstitial fluid pressure. Am J Physiol Heart Circ Physiol 287: H963–H968.

Cata JP, Weng HR, Chen JH, Dougherty PM (2006) Altered discharges of spinal wide dynamic range neurons and down-regulation of glutamate transporter expression in rats with paclitaxel-induced hyperalgesia. Neuroscience 138: 329–338.

Flatters SJL, Bennett GJ (2004) Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain 109: 150–161.

Flatters SJL, Bennett GJ (2006) Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction. Pain 122: 245–257.

Flatters SJL, Xiao WH, Bennett GJ (2006) Acetyl-L-carnitine prevents and reduces paclitaxel-induced painful neuropathy. Neurosci Lett 397: 219–223.

Garrido-Suárez BB, Garrido G, Castro-Labrada M, Merino N, Valdés O, Pardo Z, Ochoa-Rodríguez E, Verdecia-Reyes Y, Delgado-Hernández R, Godoy-Figueiredo J, Ferreira SH (2020) Anti-hypernociceptive and anti-inflammatory effects of JM-20: A novel hybrid neuroprotective compound. Brain Res Bull 165: 185-197.

Gewandter JS, Dworkin RH, Finnerup NB, Mohile NA (2017) Painful chemotherapy-induced peripheral neuropathy: lack of treatment efficacy or the wrong clinical trial methodology? Pain 158: 30–33.

Itoh Y, Sendo T, Hirakawa T, Goromaru T, Takasaki S, Yahata H, Nakano H, Oishi R (2004) Role of sensory nerve peptides rather than mast cell histamine in paclitaxel hypersensitivity. Am J Respir Crit Care Med 169: 113–119.

Jaggi AS, Singh N (2012) Mechanisms in cancer-chemotherapeutic drugs-induced peripheral neuropathy. Toxicology 291: 1–9.

Jin HW, Flatters SJL, Xiao WH, Mulhern HL, Bennett GJ (2008) Prevention of paclitaxel-evoked painful peripheral neuropathy by acetyl-L-carnitine: Effects on axonal mitochondria, sensory nerve fiber terminal arbors, and cutaneous Langerhans cells. Exp Neurol 210: 229–237.

Kidd JF, Pilkington MF, Schell MJ, Fogarty KE, Skepper JN, Taylor CW, Thorn P (2002) Paclitaxel affects cytosolic calcium signals by opening the mitochondrial permeability transition pore. J Biol Chem 277: 6504–6510.

Ko MH, Hub ME, Hsieh YL, Lan CT, Tseng TJ (2014) Peptidergic intraepidermal nerve fibers in the skin contribute to the neuropathic pain in paclitaxel-induced peripheral neuropathy. Neuropeptides 48: 109–117.

Krukowski K, Nijboer CH, Huo XJ, Kavelaars A, Heijnen CJ (2015) Prevention of chemotherapy-induced peripheral neuropathy by the small-molecule inhibitor pifithrin-μ. Pain 156: 2184–2192.

Latremoliere A, Woolf CJ (2009) Central sensitization: A generator of pain hypersensitivity by central neural plasticity. J Pain 10: 895–926.

Nuñez-Figueredo Y, Ochoa-Rodríguez E, Verdecia-Reyes Y, Carrillo- Domínguez C, Lagarto-Parra A, Ramirez-Sánchez Y, Delgado-Hernández R, Porto Verdecia M, Pardo Andreu GL (2013) Characterization of the anxiolytic and sedative profile of JM-20: a novel benzodiazepine-dihydropyridine hybrid molecule. Neurol Res 35: 804–812.

Nuñez-Figueredo Y, Pardo Andreu GL, Oliveira Loureiro S, Ganzella M, Ramírez-Sánchez J, Ochoa-Rodríguez E, Verdecia-Reyes Y, Delgado-Hernández R, Souza DO (2015) The effects of JM-20 on the glutamatergic system in synaptic vesicles, synaptosomes and neural cells cultured from rat brain. Neurochem Int 81: 41–47.

Nuñez-Figueredo Y, Pardo-Andreu GL, Ramírez-Sánchez J, Delgado-Hernández R, Ochoa-Rodríguez E, Verdecia-Reyes Y, Naal Z, Muller AP, Portela LV, Souza DO (2014b) Antioxidant effects of JM-20 on rat brain mitochondria and synaptosomes: mitoprotection against Ca^{2+}-induced mitochondrial impairment. Brain Res Bull 109: 68–76.

Nuñez-Figueredo Y, Ramírez-Sánchez J, Delgado-Hernández R, Porto-Verdecia M, Ochoa-Rodríguez E, Verdecia-Reyes Y, Marin-Prida J, González-Durruthy M, Uyemura SA, Rodrigues FP, Curti C, Souza DO, Pardo-Andreu GL (2014c) JM-20, a novel benzodiazepine–dihydropyridine hybrid molecule, protects mitochondria and prevents ischemic insult-mediated neural cell death *in vitro*. Eur J Pharmacol 726: 57–65.

Nuñez-Figueredo Y, Ramírez-Sánchez J, Hansel G, Simões Pires EN, Merino N, Valdes O, Delgado-Hernández R, Parra AL, Ochoa-Rodríguez E, Verdecia-Reyes Y, Salbego C, Costa SL, Souza DO, Pardo-Andreu GL (2014a) A novel multi-target ligand (JM-20) protects mitochondrial integrity, inhibits brain excitatory amino acid release and reduces cerebral ischemia injury *in vitro* and *in vivo*. Neuropharmacology 85: 517–527.

Polomano R, Clark U, Mannes AJ, Bennett GJ (2001) A painful peripheral neuropathy in rat produced by the chemotherapeutic drug, paclitaxel. Pain 94: 293–304.

Pittman SK, Gracias NG, Vasko MR, Fehrenbacher JC (2014) Paclitaxel alters the evoked release of calcitonin gene-related peptide from rat sensory neurons in culture. Exp Neurol 253: 146–153.

Ramírez-Sánchez J, Simões Pires EN, Meneghetti A, Hansel G, Nuñez-Figueredo Y, Pardo-Andreu GL, Ochoa-Rodríguez E, Verdecia-Reyes Y, Delgado-Hernández R, Salbego C, Souza DO (2018) JM-20 treatment after MCAO reduced astrocyte reactivity and neuronal death on peri-infarct regions of the rat brain. Mol Neurobiol 56: 502–512.

Ramírez-Sánchez J, Simões Pires EN, Nuñez-Figueredo Y, Pardo-Andreu GL, Fonseca-Fonseca LA, Ruiz-Reyes A, Ochoa-Rodríguez E, Verdecia-Reyes Y, Delgado Hernández R, Souza DO, Salbego C (2015) Neuroprotection by JM-20 against oxygen-glucose deprivation in rat hippocampal slices: Involvement of the Akt/GSK-3β pathway. Neurochem Int 90: 215–223.

Seretny M, Currie GL, Sena ES, Ramnarine S, Grant R, MacLeod MR, Colvin LA, Fallon M (2014) Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. Pain 155: 2461–2470.

Siau C, Xiao W, Bennett GJ (2006) Paclitaxel- and vincristine-evoked painful peripheral neuropathies: Loss of epidermal innervation and activation of Langerhans cells. Exp Neurol 201: 507–514.

Sibaud V, Leboeuf NR, Roche, H, Belum VR, Gladieff L, Deslandres M, Montastruc M, Eche A, Vigarios E, Dalenc F, Lacouture ME (2016) Dermatological adverse events with taxane chemotherapy. Eur J Dermatol 26: 427–443.

Sisignano M, Baron R, Scholich K, Gerd Geisslinger G (2014) Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. Nat Rev Neurol 10: 694–707.

Summers DW, DiAntonio A, Milbrandt J (2014) Mitochondrial Dysfunction Induces Sarm1-Dependent Cell Death in Sensory Neurons J Neurosci 34: 9338–9350.

Tricaud N, Park HT (2017) Wallerian demyelination: Chronicle of a cellular cataclysm. Cell Mol Life Sci 74: 4049–4057.

Ventzel L, Jensen AB, Jensen AR, Jensen TS, Finnerup NB (2016) Chemotherapy-induced pain and neuropathy: a prospective study in patients treated with adjuvant oxaliplatin or docetaxel. Pain 157: 560–568.

Weng HR, Aravindan N, Cata JP, Chen JH, Shaw ADS, Dougherty PM (2005) Spinal glial glutamate transporters downregulate in rats with taxol-induced hyperalgesia. Neurosci Lett 386: 18–22.

Wiegert JS, Bading H (2011) Activity-dependent calcium signaling and ERK-MAP kinases in neurons: A link to structural plasticity of the nucleus and gene transcription regulation. Cell Calcium 49: 296–305.

Wolf S, Barton D, Kottschade L, Grothey A, Loprinzi C (2008) Chemotherapy-induced peripheral neuropathy: Prevention and treatment strategies. Eur J Cancer 44: 1507-1515.

Xia WH, Bennett GJ (2008) Chemotherapy-evoked neuropathic pain: Abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-L-carnitine. Pain 135: 262–270.

Xia WH, Bennett GJ (2012) Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. Pain 153: 704–709.

Xiao WH, Zheng FY, Bennett GJ, Bordet T, Pruss RM (2009) Olesoxime (cholest-4-en-3-one, oxime): Analgesic and neuroprotective effects in a rat model of painful peripheral neuropathy produced by the chemotherapeutic agent, paclitaxel. Pain 147: 202–209.

AUTHOR CONTRIBUTION:

Contribution	Garrido-Suárez BB	Garrido G	Menéndez AB	Aparicio G	Valdés O	Ochoa-Rodríguez E	Verdecia-Reyes Y	Delgado-Hernández R
Concepts or ideas	x							x
Design	x							
Definition of intellectual content	x							
Literature search	x							
Experimental studies	x		x		x	x	x	
Data acquisition	x							
Data analysis	x			x				
Statistical analysis	x							
Manuscript preparation	x	x						
Manuscript editing	x	x						
Manuscript review	x	x	x	x	x	x	x	x

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