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ORIGINAL ARTICLE

Involvement of Endothelin Receptors in Peripheral Sensory Neuropathy Induced by Oxaliplatin in Mice

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Abstract

The aim of this study was to evaluate the participation of the endothelin ET_A and ET_B receptors and the effects of bosentan in oxaliplatin-induced peripheral sensory neuropathy (OIN) in mice. Adult male Swiss mice received 1 mg/kg of oxaliplatin intravenously, twice a week for 5 weeks. Dorsal root ganglia (DRG) and spinal cords were removed for evaluation of the endothelin ET_A and ET_B receptor expression. Afterwards, selective (BQ-123 and BQ-788; 10 nmol in 30 μL, intraplantarly) and non-selective (bosentan, 100 mg/kg, orally) antagonists were administered in order to evaluate the involvement of the endothelin receptors in OIN. Mechanical and thermal nociception tests were performed once a week for 56 days. Oxaliplatin induced mechanical and thermal hypersensitivity and increased the endothelin ET_A receptor expression in both the DRG and spinal cord ($P < 0.05$). Endothelin ET_B receptor expression was increased in the DRG ($P < 0.05$) but not in the spinal cord. Both endothelin ET_A and ET_B receptor selective antagonists partially prevented mechanical hyperalgesia in mice with OIN ($P < 0.05$). Moreover, bosentan prevented mechanical and thermal hypersensitivity in oxaliplatin-treated mice ($P < 0.05$). In conclusion, both endothelin ET_A and ET_B receptors seem to be involved in the OIN in mice and they should be considered possible targets for the management of this clinical feature.

Keywords Pain · Neuropathic pain · Oxaliplatin · Endothelin receptors

Introduction

Oxaliplatin (OXL), a third-generation platinum agent, has a wide spectrum of antitumor activity in human cancer cell lines (Kelland 2007; Argyriou et al. 2008). It is usually used in combination with 5-fluorouracil and leucovorin in FOLFOX and FOLFOXIRI protocols, being one of the first-line regimens for the treatment of metastatic colorectal cancer (de Gramont et al. 2000).

The main OXL-related dose-limiting side effect is neurotoxicity, reducing the treatment tolerability, which might lead to treatment regimen change/suppression (Argyriou et al. 2008). OXL induces a peripheral sensory neuropathy, which may present acute (with symptoms such as perioral and distal paresthesias) (Schiff et al. 2009) or chronic symptoms (presenting distal mechanical and thermal hyperalgesia/allodynia) (Argyriou et al. 2008; Azevedo et al. 2013). The OXL-induced neurotoxicity has a difficult management, since the therapeutic approaches used for the treatments of neuropathic pain, such as antidepressants, anticonvulsants, opioids, topical lidocaine, and infusion of calcium and magnesium, among others, are not fully effective in reducing its symptoms (Baron et al. 2010).

Endothelin-1 (ET-1) is a 21-residue potent vasoactive peptide that is known to be secreted by many cancer cell lines, such as pancreatic, prostate, and breast cancers (Kusuhara et al. 1990; Nakayama et al. 1998). ET-1 acts through two

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different receptors, ET_A and ET_B. The endothelin ET_B receptor is mainly found in non-neural tissues, for instance, endothelial cells, macrophages, and keratinocytes, while the endothelin ET_A receptor is mainly found in the nociceptors themselves (Smith et al. 2014), even though both receptors are expressed in the dorsal root ganglia (DRG) (Plant et al. 2007). Studies have shown that ET-1 is capable of inducing pain or overt nociception in animals and in humans, regardless of its vasoconstrictor effect (Ferreira et al. 1989; Davar et al. 1998; Piovezan et al. 2004), probably due to direct activation of the primary nociceptive afferent (Gokin et al. 2001; Zhou et al. 2001; Zhou et al. 2002). However, some studies (Khodorova et al. 2003; Khodorova et al. 2009) have reported that ET-1 might exert an anti-nociceptive effect, emphasizing a possible dual effect of ET-1 and its receptors in models of pain.

The aim of this study was to evaluate the role of endothelin ET_A and ET_B receptors in the OXL-induced peripheral sensory neuropathy in mice, as well as the effects of bosentan, an unspecific antagonist of the endothelin receptors, as a possible therapeutic agent for this side effect.

Materials and Methods

Sample Size and Animals

Sample size was calculated considering the animal to be the unit of the study and the variation of the paw withdrawal threshold to be the primary outcome, considering the same sample size methodology previously used by our group (Pereira et al. 2018). Thus, 7 animals were used in each experimental group.

Seventy-seven male Swiss (*Mus musculus*) mice, weighing 25–30 g, from the Central Animals Facility of the Federal University of Ceará, randomly allocated in the experimental groups, were used in the present study. The mice were placed in appropriate cages in a silent room under a controlled temperature (22 ± 2 °C) and a 12-h light/dark cycle, with free access to solid food and water. The experiments were executed in our own animal facilities in conformity with the local guidelines on the welfare of experimental animals and with the approval of the Ethics Commission in Animal Research of the Federal University of Ceará (protocol number 75/2012).

Peripheral Sensory Neuropathy Induced by OXL

The peripheral sensory neuropathy model induced by OXL developed by Azevedo et al. (2013) was used, which was based on the method of Ling et al. (2007). OXL (Sigma-Aldrich®, St. Louis, MO, USA) was diluted in a 5% glucose solution (Dinâmica Química Contemporânea Ltda., Rio de Janeiro, RJ, Brazil) and injected twice a week in the lateral vein of the mice's tail in a dose of 1 mg/kg, with a total of nine injections. The vehicle group was injected intravenously with 5% glucose solution. After nine injections, the mice also were assessed by mechanical and thermal nociceptive tests until the 56th experimental day, with the purpose of evaluating long-term effects of the OXL administrations.

Immunofluorescence

On the 28th (end of the injection period) and the 56th days (end of the experimental period), the mice ($n = 7$) were profoundly anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) (König do Brasil Ltda., Mairinque, SP, Brazil) and xylazine (10 mg/kg) (König do Brasil Ltda., Mairinque, SP, Brazil) and intracardially perfused with 40 mL of sterile saline solution and 40 mL of 4% paraformaldehyde (PFA) solution (Sigma-Aldrich®, St. Louis, MO, USA). After the perfusion, the L5 DRG and a portion of the spinal cord (located between L4 and L6) were extracted and placed in 4% PFA for 2 hours, followed by an immersion in 30% sucrose solution (Dinâmica Química Contemporânea Ltda., Rio de Janeiro, RJ, Brazil) for 2 days. After cryoprotection, the tissues were embedded in Tissue-Tek O.C.T. compound (Sakura®, Netherlands) and stocked in a temperature of − 80 °C for further analyses. Five serial sections of each sample were made in a thickness of 10 μm (DRG) or 20 μm (spinal cord) in a cryostat (Leica CM1850, Leica, Wetzlar, Germany).

For immunofluorescence, the sections were fixed in methanol (Vetec Química Fina Ltda., Duque de Caxias, RJ, Brazil) and the antigenic recovery was executed in 0.1 M (pH 6.0) citrate buffer at 95 °C. Then, unspecific sites were blocked with 5% bovine serum albumin (Sigma-Aldrich®, St. Louis, MO, USA) and 0.3 M glycine (Sigma-Aldrich®, St. Louis, MO, USA). The sections were incubated overnight with primary antibody rabbit anti-ET_A (Sigma-Aldrich®, St. Louis, MO, USA), rabbit anti-ET_B (Sigma-Aldrich®, St. Louis, MO, USA), or mouse anti-glutamine synthetase (Merck Millipore®, Billerica, MA, USA) at a dilution of 1:200, 1:100, and 1:200, respectively. Then, the sections were incubated with secondary goat anti-rabbit IgG Alexa Fluor 594 (Invitrogen®, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) or goat anti-mouse IgG Alexa Fluor 633 (Invitrogen®, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA), using a dilution of 1:400. Afterwards, sections were incubated with a NeuN antibody conjugated with Alexa Fluor 488 (Merck Millipore®, Billerica, MA, USA), using a dilution of 1:100, for the labeling of neuronal cell bodies. For nuclear labeling, the slides were incubated with DAPI (4 μL in 200 mL of PBS) for 30 min. Finally, the slides were prepared (ProLong Gold Antifade Mountant, Invitrogen®, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) and photographed

using a confocal microscope (Zeiss LSM 710, Carl Zeiss, Jena, Germany).

In the photomicrographs, the quantification of the fluorescent area was performed using an image analysis software (Fiji ImageJ, National Institutes of Health, Washington, DC, USA). This quantification was made by a blind researcher through differentiation of the fluorescent areas by the higher color saturation associated with the fluorescence (red or green). The lower and upper limits required for the definition of fluorescent and non-fluorescent pixels were previously chosen by a color threshold. The results of the quantification were shown in percentage, which was calculated by positive fluorescence compared with NeuN fluorescence.

Evaluation of the Involvement of Endothelin Receptors in OXL-Induced Peripheral Sensory Neuropathy

The evaluation of the involvement of the endothelin receptors in OXL-induced peripheral sensory neuropathy was performed using endothelin ET_A and ET_B receptor antagonists. The selective antagonists BQ-123 (endothelin ET_A receptor antagonist) and BQ-788 (endothelin ET_B receptor antagonist) were diluted in phosphate-buffered saline (PBS) and injected (10 nmol; 30 μL) intraplantarly in the right hind paw ($n = 7$) of the mice 30 min before each OXL injection (Motta et al. 2009a; Motta et al. 2009b; Yoshino et al. 2018). In the vehicle group, PBS (30 μL) was injected to the right hind paw ($n = 7$). Additionally, we aimed to test the effects of bosentan (Tracleer®, Actelion Pharmaceuticals do Brasil Ltda., Rio de Janeiro, RJ, Brazil), an orally delivered unspecific endothelin ET_A and ET_B receptor antagonist used for the treatment of pulmonary hypertension, which was dissolved in 2% carboxymethylcellulose (Labsynth® Produtos para Laboratórios Ltda., Diadema, SP, Brazil) and orally administered (100 mg/kg) 30 min before each OXL injection ($n = 7$) (Serafim et al. 2015). Similarly, the vehicle group received only 2% carboxymethylcellulose orally ($n = 7$).

Behavioral Tests

Mechanical Plantar Hyperalgesia Test

The intensity of plantar mechanical hyperalgesia was assessed by each mouse's sensitivity threshold to a mechanical stimulus caused by the gradual pressure exerted punctually by the tip of a rigid filament coupled to a digital analgesimeter apparatus (Electronic von Frey, Insight®, Ribeirão Preto, SP, Brazil), which registers the exerted pressure in grams, as previously performed by our group (Azevedo et al. 2013; Pereira et al. 2018). An inclined mirror was placed below the grid. The Electronic von Frey apparatus recorded the pressure that caused a reaction of paw flexion followed by a flinch after paw withdrawal. Plantar mechanical hyperalgesia was evaluated by a blind calibrated researcher (Cunha et al. 2004).

Cold Allodynia Test

The tail immersion test in cold water was executed using the non-noxious temperature of 10 °C by a blind experimenter (Necker and Hellon 1978; Ling et al. 2007). For this test, the mice's tails were immersed in cold water until withdrawal by the animals. The duration of the immersion was recorded in seconds, using a cut-off time of 120 s. One week before the beginning of the experiment, the mice were adapted to the handling by the researchers. All behavioral tests were carried out between 1 and 3 p.m.

Statistical Analysis

The results were expressed as mean ± standard deviation of the mean (SD). Data were tested for normality and homoscedasticity. The verification of the statistical differences between the experimental groups in the behavioral tests was performed using two-way analysis of variance (two-way ANOVA) followed by the Bonferroni test. The unpaired t test was used, for other results. Seven animals were used per experimental group. The level of significance was set at $P < 0.05$. GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, USA) was used for the analyses.

Results

Effect of OXL on Endothelin ET_A Receptor Expression in the DRG and Dorsal Horn of the Spinal Cord

OXL increased endothelin ET_A receptor expression in neuronal cells from the DRG of mice on the 28th (Figs. 1a and 2) and 56th (Figs. 1b and 2) days in comparison with the vehicle-treated group (4.28-fold and 14.33-fold higher, respectively; $P < 0.05$). Furthermore, endothelin ET_A receptor expression in non-neuronal cells in the DRG of mice was observed on the 28th day (Fig. 2). Therefore, a new immunofluorescence assay was performed in order to identify the cellular type that was expressing the ET_A receptor. It was found that satellite glial cells (glutamine synthetase positive) were responsible for this extra-neuronal expression (Fig. 3).

In the dorsal horn of the spinal cord, there was an increase in the endothelin ET_A receptor expression in neuronal cells on the 28th day, compared with the group treated with vehicle (5.38-fold higher; Figs. 1c and 4) ($P < 0.05$). Such increase was not observed on the 56th day (Figs. 1d and 4).

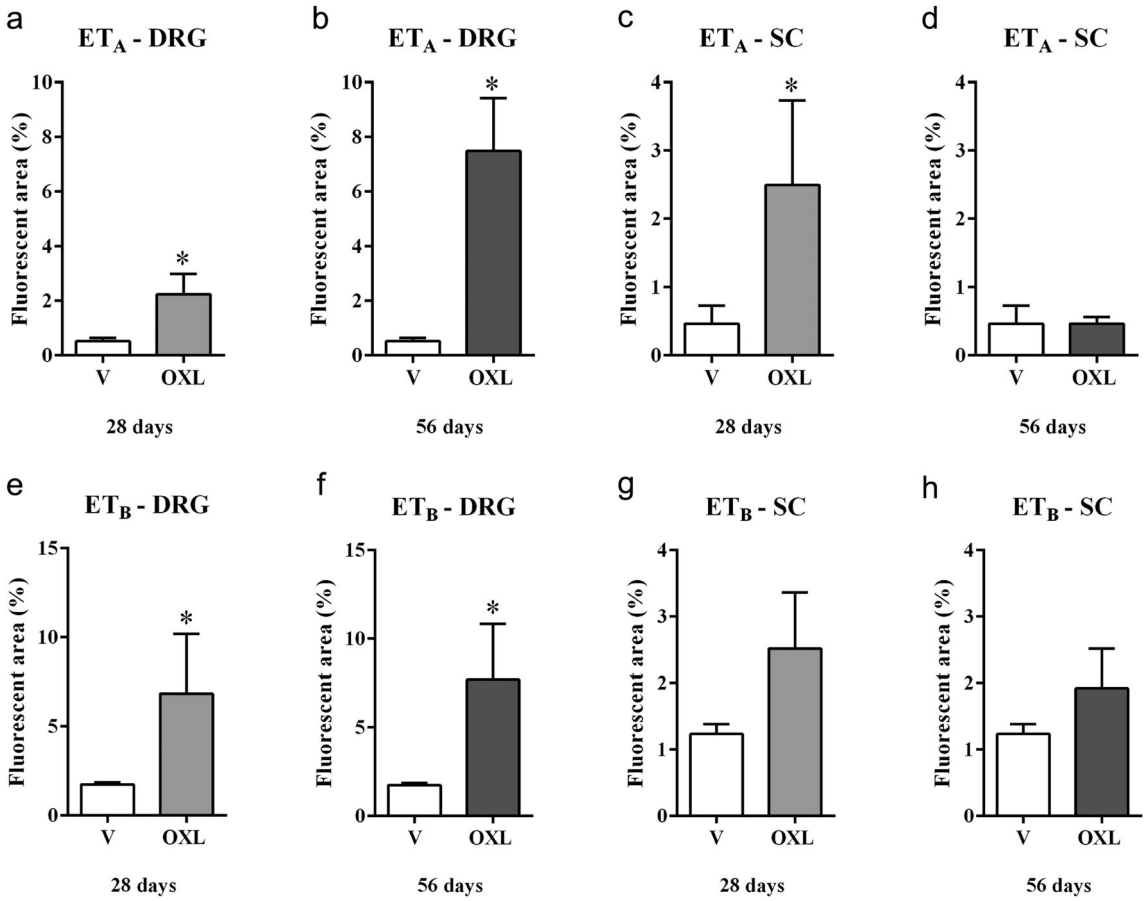

Fig. 1 Quantification of the fluorescent areas of the endothelin ET_A and ET_B receptor expression in the DRG and the dorsal horn of the spinal cord of the mice. The percentage of the positive fluorescent area in relation to NeuN (neuronal marker) expression is represented as mean ± SD in the bar graphs. *$P < 0.05$ versus vehicle (unpaired *t* test). DRG, dorsal root ganglia; ET_A, endothelin ET_A receptor; ET_B, endothelin ET_B receptor; OXL, oxaliplatin; SC, spinal cord; V, vehicle

Effect of OXL on Endothelin ET_B Receptor Expression in the DRG and Dorsal Horn of the Spinal Cord

OXL significantly increased endothelin ET_B receptor expression in neuronal cells from the DRG of mice on the 28th (Figs. 1e and 5) and 56th (Figs. 1f and 5) days, compared with the vehicle group (3.91-fold and 4.41-fold higher, respectively; $P < 0.05$). The expression of the endothelin ET_B receptor in the spinal cord was not influenced by the administration of OXL (Figs. 1g, h and 6).

Effect of BQ-123 (Endothelin ET_A Receptor Antagonist) and BQ-788 (Endothelin ET_B Receptor Antagonist) on the Variation of the Mechanical Nociceptive Threshold in Mice Injected with OXL

The variation of the mechanical nociceptive threshold was evaluated in the hind paws of mice that received intravenous injections of OXL (1 mg/kg). It was observed that OXL increased the variation of the mechanical nociceptive threshold from the 7th to the 56th day (Fig. 7; $P < 0.05$). Intraplantar administration of BQ-123 (10 nmol; 30 μL) partially reverted nociceptive response induced by OXL between the 14th day and the 56th day ($P < 0.05$), while BQ-788 (10 nmol; 30 μL) prevented it from the 28th to the 56th day (Fig. 7; $P < 0.05$).

Effect of Bosentan on the Variation of the Mechanical Nociceptive Threshold in Mice Injected with OXL

It was observed that OXL increased the variation of the mechanical nociceptive threshold from the 28th to the 56th day ($P < 0.05$) and bosentan (100 mg/kg) partially prevented nociceptive response induced by the administration of OXL between days 28 and 56 (Fig. 8a; $P < 0.05$).

Effect of Bosentan on the Cold Nociceptive Threshold in Mice Injected with OXL

In the OXL group, there was a decrease in the tail withdrawal time (s) from the 7th to the 56th day ($P < 0.05$). The administration of bosentan (100 mg/kg) partially prevented the

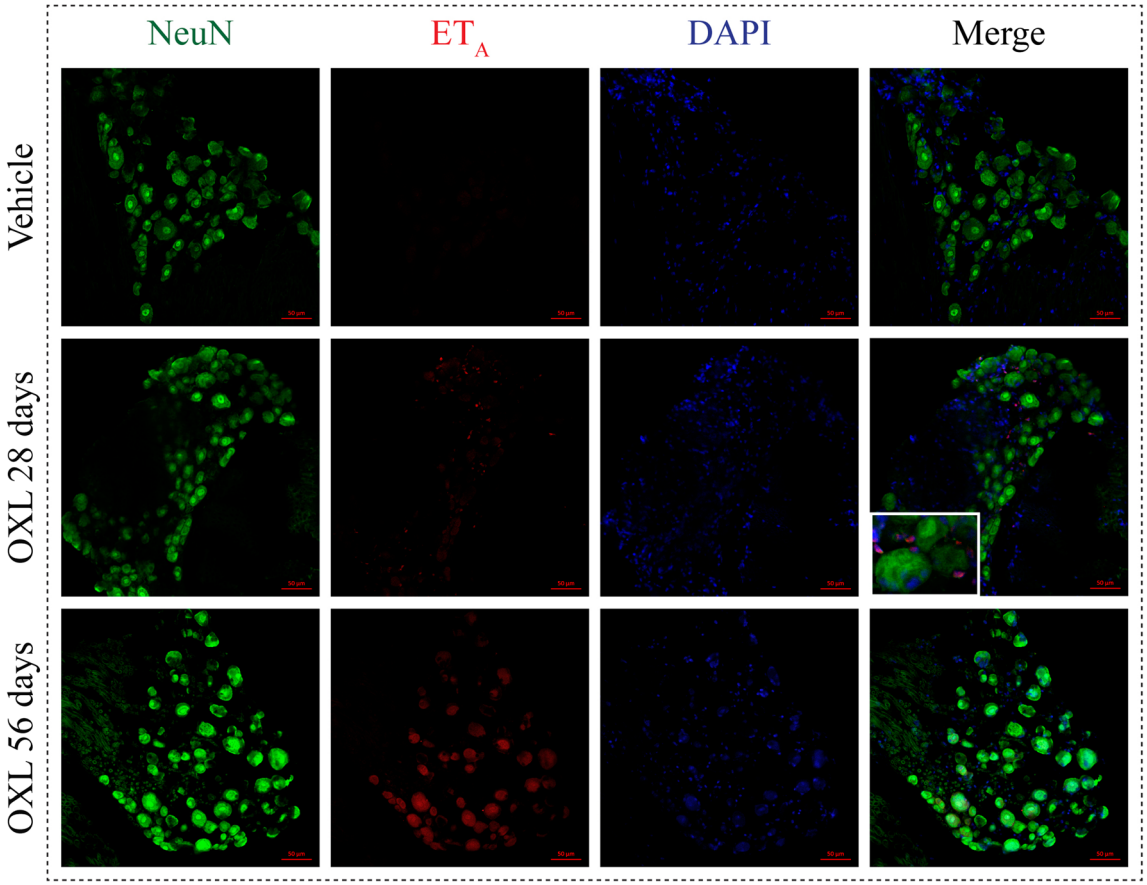

Fig. 2 Endothelin ET_A receptor expression induced by OXL in the DRG of mice. Green: NeuN (neuronal marker); red: endothelin ET_A receptor; blue: DAPI (nuclear marker). In the white border rectangle, a greater magnification of non-neuronal cells is shown presenting positive fluorescence to c-Fos. ×200 magnification. ET_A, endothelin ET_A receptor; OXL, oxaliplatin

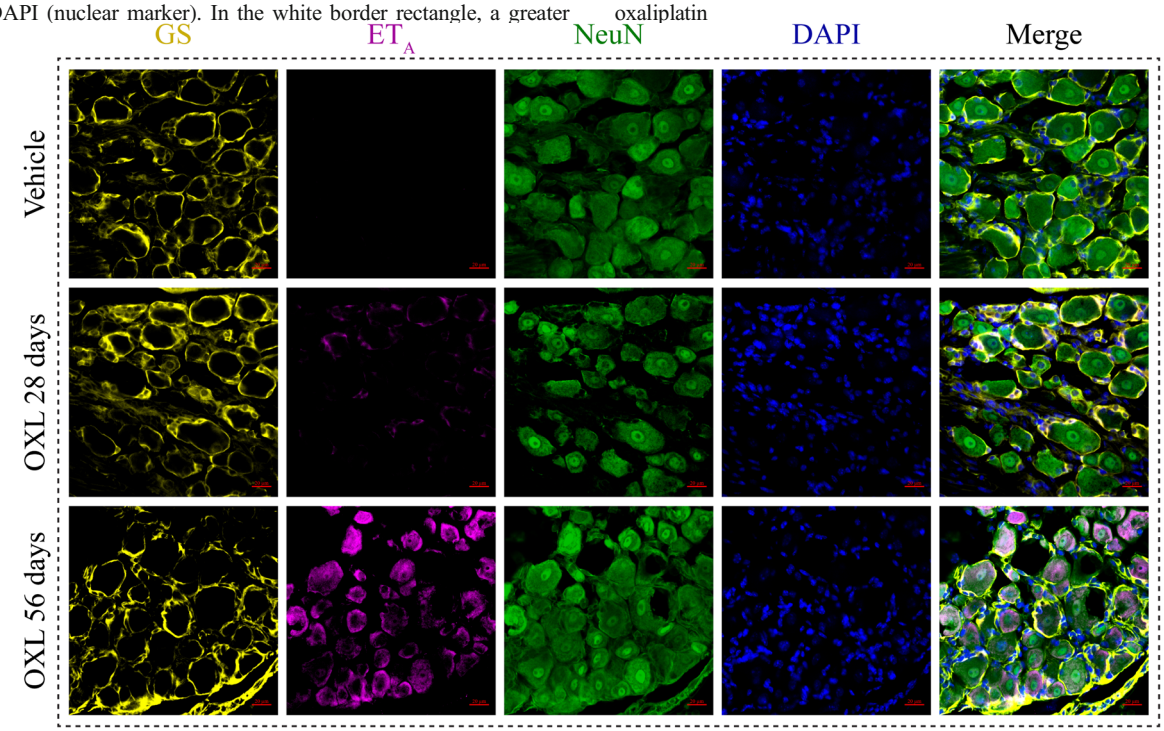

Fig. 3 Endothelin ET_A receptor expression induced by OXL in satellite glial cells in the DRG of mice. Yellow: glutamine synthetase (satellite glial cell marker); magenta: endothelin ET_A receptor; green: NeuN (neuronal marker); blue: DAPI (nuclear marker). ×400 magnification. ET_A, endothelin ET_A receptor; GS, glutamine synthetase; OXL, oxaliplatin

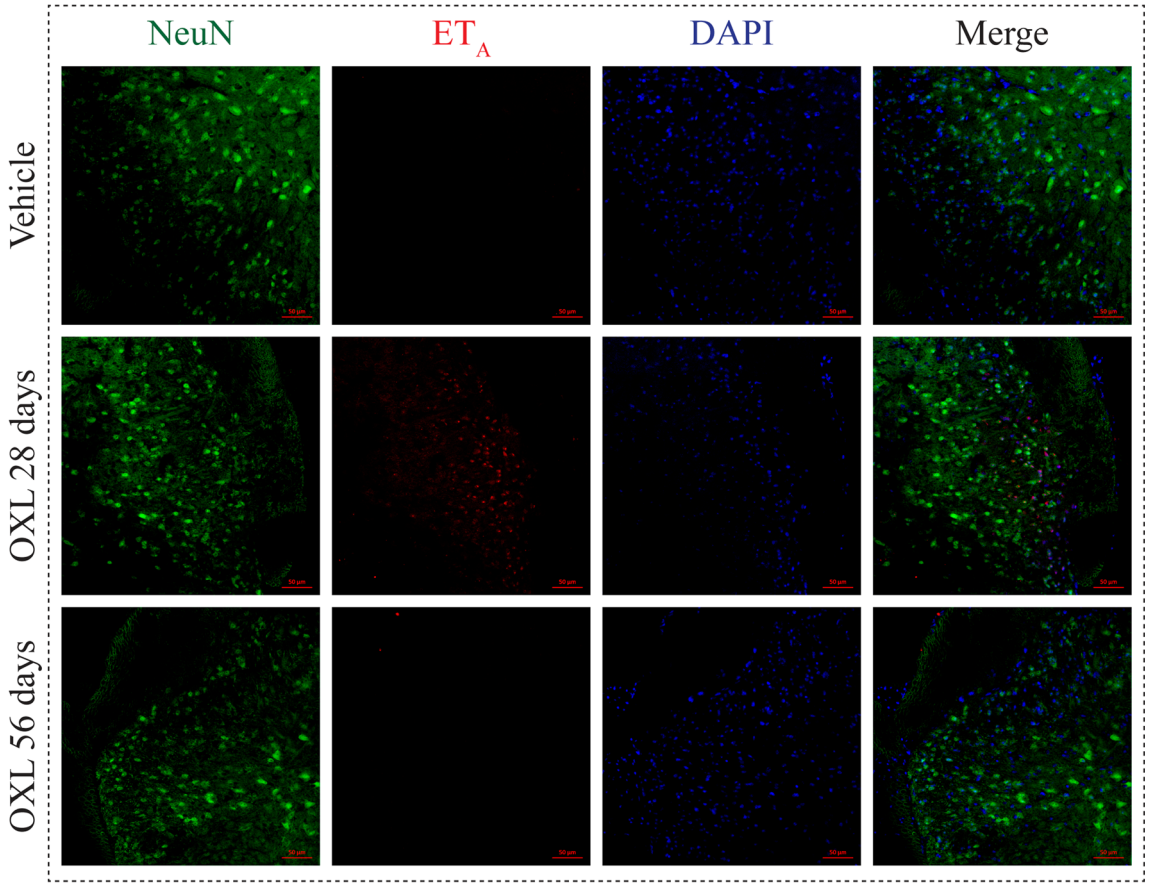

Fig. 4 Endothelin ET_A receptor expression induced by OXL in the dorsal horn of the spinal cord of mice. Green: NeuN (neuronal marker); red: endothelin ET_A receptor; blue: DAPI (nuclear marker). ×200 magnification. ET_A, endothelin ET_A receptor; OXL, oxaliplatin

aforementioned decrease induced by OXL between days 7 and 56 (Fig. 8b; $P < 0.05$).

Discussion

Since the neurotoxicity, expressed as peripheral sensory neuropathy, is the main dose-limiting side effect of OXL treatment, the mechanisms underlying this condition, as well as its therapeutic approaches, have been extensively studied (Zedan et al. 2014). In this study, we aimed to assess a possible participation of endothelin ET_A and ET_B receptors in OXL-induced peripheral sensory neuropathy by evaluating their expression in the neural tissues and the mechanical and thermal nociception thresholds after their inhibition in mice. Moreover, we aimed to evaluate those receptors as possible targets for reducing the establishment of OXL-induced sensory neuropathy. Here, we found that both receptors may play a role in the increased mechanical sensitivity induced by OXL, since their expression seems to be altered in neural tissues after OXL injection. Furthermore, it was shown that the selective peripheral blockage of both receptors, as well as the systemic mutual blocking, led to reduced mechanical and cold sensitivity.

ET-1 is known to evoke hyperpolarizing shifts in the tetrodotoxin-resistant Na^+ channels on sensitive neurons, which solely depolarizes sensory fibers, leading to the activation of nociceptors (Zhou et al. 2002; Plant et al. 2007) and inducing rapid and long-lasting discharges of non-myelinated C fibers (Gokin et al. 2001). This mechanism is probably responsible for the mechanical and thermal hyperalgesia/allodynia observed following ET-1 experimental injection (Ferreira et al. 1989; Raffa et al. 1991). Interestingly, both endothelin ET_A and ET_B receptors seem to be involved in these outcomes in both inflammatory and neuropathic pain (Baamonde et al. 2004; Chichorro et al. 2006a, 2006b ; Khodorova et al. 2009). It is important to emphasize that ET-1-induced hyperalgesia happens regardless of its vasoactive effect, sympathetic activation, or prostaglandin production (da Cunha et al. 2004). To the best of our knowledge, this is the very first evidence that OXL leads to an increased expression of endothelin ET_A and ET_B receptors in the DRG and in the dorsal horn of the spinal cord in mice. Recently, our group has shown that OXL administration leads to a cumulative neural damage (Pereira et al. 2018). It was presented here

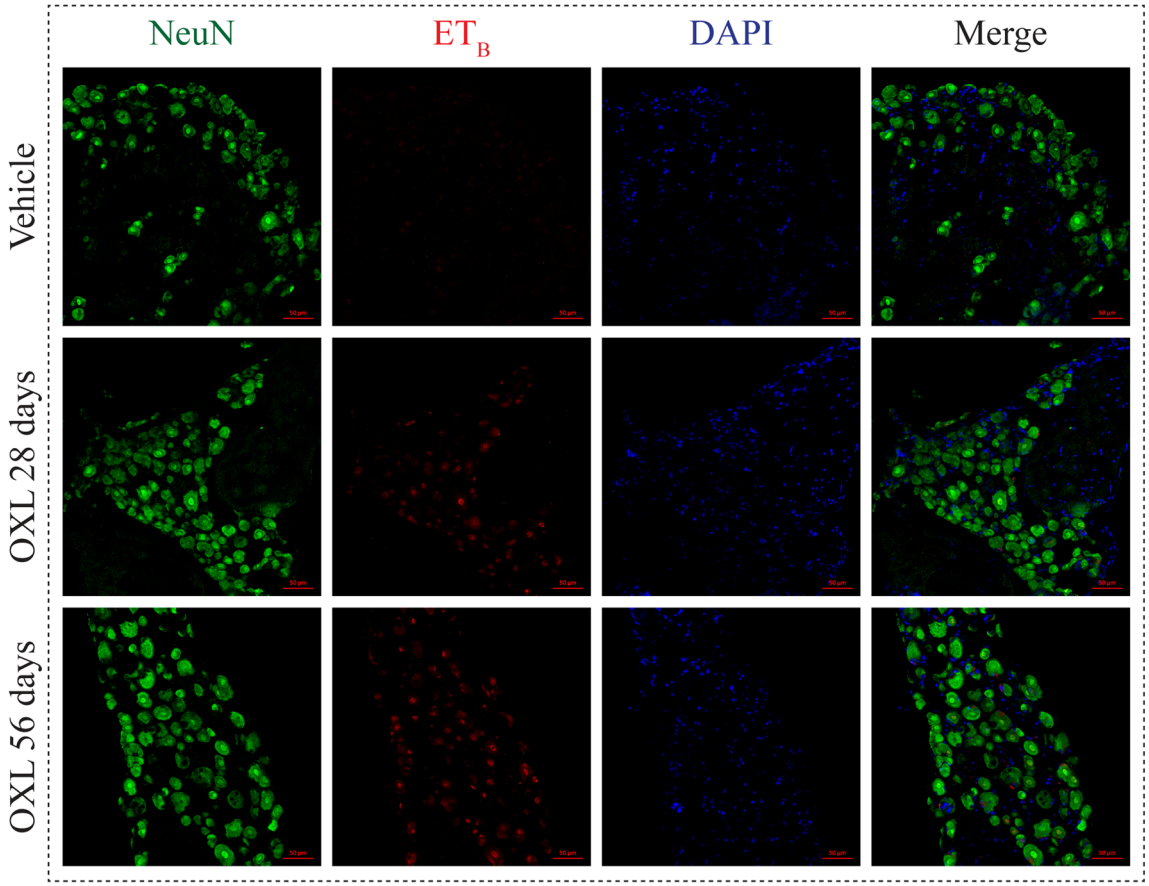

Fig. 5 Endothelin ET_B receptor expression induced by OXL in the DRG of mice. Green: NeuN (neuronal marker); red: endothelin ET_B receptor; blue: DAPI (nuclear marker). ×200 magnification. ET_B, endothelin ET_B receptor; OXL, oxaliplatin

that ET_A and ET_B expression levels in the DRG are still increased even after the OXL injection period, which does not happen in the central nervous system. Since damaged nerves present high levels of ET-1, ET_A and ET_B mRNAs (Klass et al. 2005; Werner et al. 2010), the increased expression of the endothelin receptors in the neural tissues, found in this study, might be one of the reasons for this long-term mechanical and thermal hyperalgesia/allodynia induced by OXL.

Another interesting finding was that, by the end of the OXL injection period (28th experimental day), it was possible to find ET_A expression in satellite glial cells. In fact, the presence of the endothelin receptors in those cells has been reported in the DRG of both naïve animals and animals with neuropathic pain (Pomonis et al. 2001; Chichorro et al. 2006a). It has been demonstrated that there is a robust intraganglionic interaction between neurons and satellite glial cells, in a way that these cells are activated during distal neuronal damage, possibly inducing inflammatory and/or regenerative responses (Christie et al. 2015). By activating these receptors in satellite cells, the presence of ET-1 induces an increase in the intracellular concentration of Ca^{2+}, which might facilitate the secretion of important mediators for the neuron-glia communication (Feldman-Goriachnik and Hanani 2017).

In this study, we have found that intraplantar injection of ET_A and ET_B antagonists separately led to a significant lower variation in the mechanical nociceptive threshold in mice treated with OXL. While the modulating role of the endothelin ET_A receptor in nociception has been widely accepted, there is still some dichotomy in the ET_B actions in models of inflammatory and neuropathic pain (Baamonde et al. 2004; Piovezan et al. 2004; Verri et al. 2004; Khodorova et al. 2009). This uncertainty about the roles played by ET_B receptors in pain processing relies on two main divergent theories: (1) the activation of these receptors leads to a release of interleukin-6 and tumor necrosis factor-α by peripheral tissues or non-neuronal glial cells, producing hyperalgesia (Baamonde et al. 2004; Khodorova et al. 2009), and (2) selective activation of the endothelin ET_B receptor blocks voltage-gated sodium current and potentiates outward transient potassium current, leading to an anti-nociceptive effect (Mule et al. 2017). In fact, the extent of the influence of the endothelin ET_B receptor in neuropathic pain seems to depend on the studied experimental model. Endothelin ET_B receptor antagonism succeeded in reducing mechanical allodynia in trigeminal nerve injury (Chichorro et al. 2006b) and in chronic nerve constriction (Klass et al. 2005; Werner et al. 2010); however, in a model

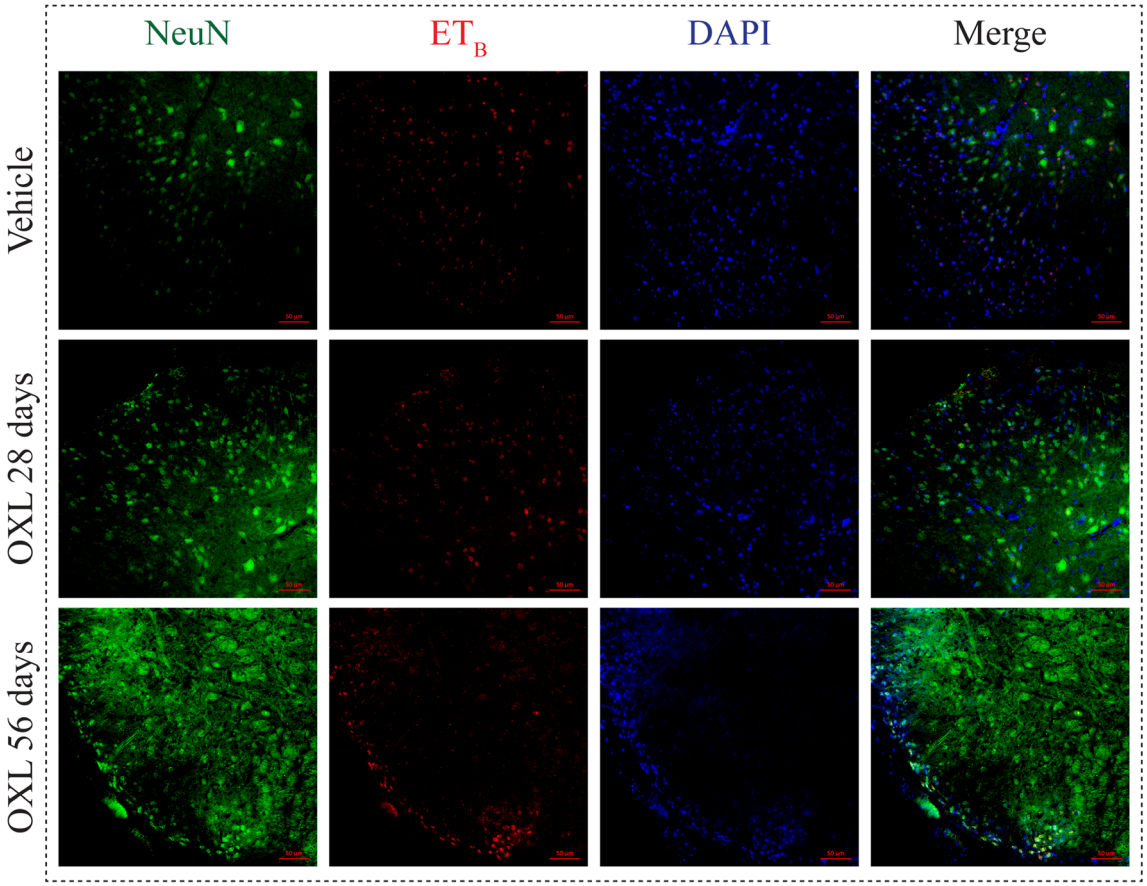

Fig. 6 Endothelin ET_B receptor expression induced by OXL in the dorsal horn of the spinal cord of mice. Green: NeuN (neuronal marker); red: endothelin ET_B receptor; blue: DAPI (nuclear marker). ×200 magnification. ET_B, endothelin ET_B receptor; OXL, oxaliplatin

of complex regional pain syndrome type I, intraplantar administration of an ET_B antagonist increased the nociceptive behaviors (Millecamps et al. 2010). It is important to stress that this research brings the first evidence that endothelin ET_B

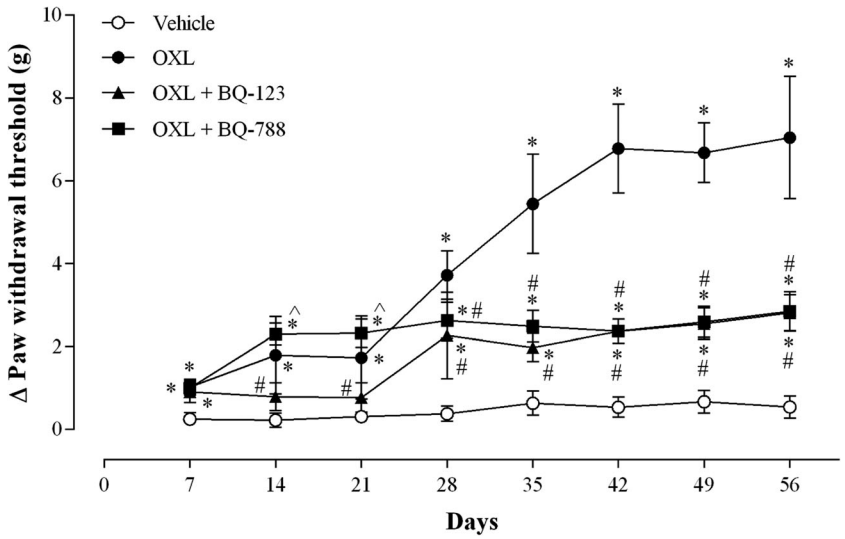

Fig. 7 Effect of BQ-123 (10 nmol; 30 μL) and BQ-788 (10 nmol; 30 μL) on the mechanical nociceptive variation of mice subjected to peripheral sensory neuropathy induced by OXL. Results are presented as mean ± SD. $^{*}P < 0.05$ versus vehicle; $^{\#}P < 0.05$ versus OXL; $^{\wedge}P < 0.05$ versus BQ-123 (two-way ANOVA followed by the Bonferroni post-test). BQ-123, endothelin ET_A receptor antagonist; BQ-788, endothelin ET_B receptor antagonist; OXL, oxaliplatin; PBS, phosphate-buffered saline

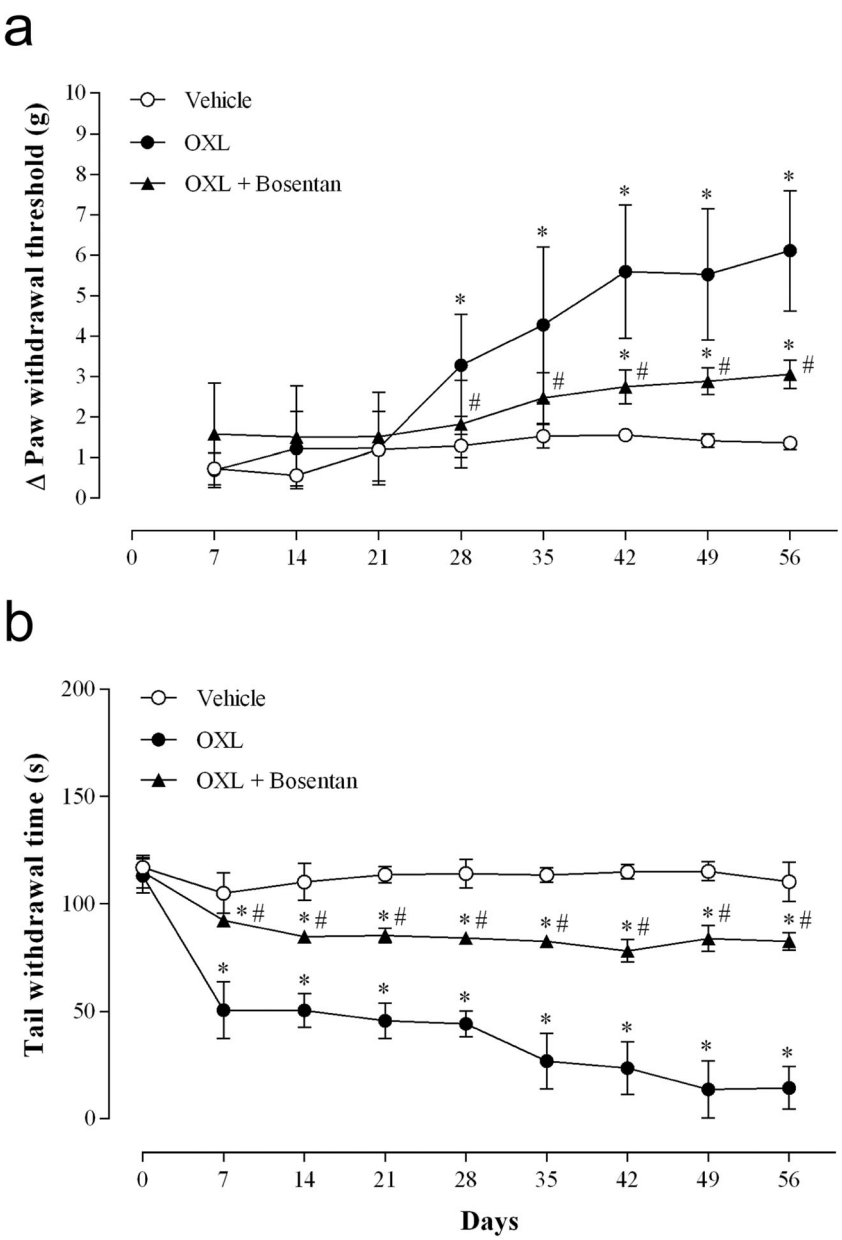

Fig. 8 Effect of bosentan on the behavioral tests of mice subjected to peripheral sensory neuropathy induced by OXL. **a** von Frey electronic test and **b** tail immersion test in cold water. Results are presented as mean ± SD. $^{*}P < 0.05$ versus vehicle; $^{\#}P < 0.05$ versus OXL (two-way ANOVA followed by the Bonferroni post-test). CMC, carboxymethylcellulose; OXL, oxaliplatin

receptor antagonism reduces mechanical nociception in chemotherapy-induced sensory neuropathy.

Bosentan is an ET_A and ET_B non-selective antagonist used for the treatment of pulmonary hypertension, which has been shown capable of reducing nociception in several models of inflammatory pain, such as superoxide anion-induced pain, antigen-induced monoarthritis, and zymosan- or carrageenan-induced inflammatory arthritis (De-Melo et al. 1998; Conte Fde et al. 2008; Imhof et al. 2011; Serafim et al. 2015). The mechanism underlying this anti-nociceptive effect in inflammatory pain seems to be related to a reduced production of tumor necrosis factor-α, interleukin-1β, leukotriene B_4, and CXCL-1 and an increased production of interleukin-10 in both peripheral tissues and neural tissues (Conte Fde et al. 2008; Serafim et al. 2015). Nevertheless, it is not clear if these effects are dependent on ET_B alone (Imhof et al. 2011) or both ET_A and ET_B receptors (Conte Fde et al. 2008). Here, we show the first evidence that bosentan is capable of partially reverting mechanical and thermal hyperalgesia/allodynia in chemotherapy-induced neuropathy. Different from the role of endothelin receptors in inflammatory pain, the effects of mutual or specific blockage of those receptors in reducing mechanical hyperalgesia in neuropathic pain are still fairly obscure, since it is not clear if it is due to the sole blockage of ET_A (Forner et al. 2016) or ET_B (Chichorro et al. 2006a) or even the blockage of both receptors (Klass et al. 2005; Werner et al. 2010). However, both endothelin ET_A and ET_B receptors seem to be involved in thermal

hyperalgesia/allodynia in neuropathic affections (Chichorro et al. 2006b). Recently, Uchida et al. (2018) showed, in an observational study, that the inhibition of the renin-angiotensin system might lead to a preventive effect of the peripheral sensory neuropathy induced by OXL. The renin-angiotensin-aldosterone system and the endothelin system work synergistically for a more potent vasoactive mechanism (Lee et al. 1990; Johnston 1992; Masaki 1995), and, interestingly, the inhibition of ET-1 receptors seems to reduce the activity of the renin-angiotensin system (Rossi et al. 1999). This might be a possible mechanism for the reduced neurotoxicity observed in our data.

Regardless of its effects on chemotherapy-induced neuropathy, patients with colorectal cancer might benefit from the modulation of the endothelin signaling. Those patients showed increased serum and tumor ET-1 expression, irrespective of the tumor staging or prognosis (Peeters et al. 2000; Hoosein et al. 2007). This excessive production of ET-1 associated with the overexpression of the endothelin ET_A receptor, also found in those tumors, might lead to increased angiogenesis and desmoplasia and further tumor progression (Grant et al. 2007; Hoosein et al. 2007), probably in consequence of the activation of YAP/TAZ, transcription coactivators of the Hippo tumor suppressor pathway (Wang et al. 2017). Nie et al. (2014) showed that the activation of endothelin ET_A receptors leads to in vitro cisplatin resistance, increased cell survival, and cell invasion induced by matrix metalloproteinase-2. In vivo, the activation of the aforementioned receptor induced liver metastasis of colon cancer (Nie et al. 2014). Furthermore, it was shown that ET_A antagonists reduced cell proliferation and migration and collagen gel contraction in strains of colorectal cancer cells, while ET_B antagonists did not prevent cell proliferation (Haque et al. 2013). Thus, since OXL is the first line in the treatment of colorectal cancer, endothelin receptors should be considered possible targets for adjuvant treatment for those patients, reducing the neurotoxic effects of OXL and possibly preventing metastasis and tumor proliferation.

Other than the inherent limitations of experimental studies in animals, further investigations should be performed in order to properly understand the mechanisms underlying the activation of the endothelin receptors in the neurotoxic effects of OXL. Moreover, other possible routes of administration of endothelin receptor antagonists should be more thoroughly studied, since it has been reported that the activation of endothelin receptors followed by intrathecal ET-1 administration seems to induce an opioid-dependent mechanical and thermal analgesia, via the activation of L-type calcium channels (Kamei et al. 1993; Yamamoto et al. 1994; Hung et al. 2012). Additionally, systemic side effects of bosentan should be taken into account while treating cancer patients, such as possible liver damage, edema, reduced hemoglobin, and pulmonary veno-occlusive disease (Dhillon 2009; Dhillon and Keating 2009).

In conclusion, endothelin signaling seems to play an important role in the development and maintenance of the OXL-induced peripheral sensory neuropathy, mainly expressed as mechanical hyperalgesia and thermal allodynia, symptoms partially reverted by the systemic administration of bosentan. Therapeutic approaches or adjunctive therapy targeting endothelin ET_A and ET_B receptors should be considered for patients with colorectal cancer, in order to reduce this dose-limiting side effect, as well as possibly reducing the possibility of distant metastasis.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted (protocol number 75/2012).

Abbreviations BQ-123, Endothelin ET_A receptor antagonist; BQ-788, Endothelin ET_B receptor antagonist; CMC, Carboxymethylcellulose; DRG, Dorsal root ganglia; ET-1, Endothelin-1; ET_A, Endothelin ET_A receptor; ET_B, Endothelin ET_B receptor; GS, Glutamine synthetase; OIN, Oxaliplatin-induced peripheral sensory neuropathy; OXL, Oxaliplatin; PFA, Paraformaldehyde; SC, Spinal cord

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