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Sexual dimorphism in vincristine-induced neuropathic pain, effect of prokineticin antagonism

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ABSTRACT

Vincristine (VCR) use is often related to neuropathy and growing evidence suggest that several factors, including sex, may influence its development. We addressed VCR-induced neuropathic pain in male and female mice in relation to neuroinflammation and prokineticin (PK) system activation in the main stations involved in pain transmission: sciatic nerve, dorsal root ganglia and spinal cord. We also evaluated the efficacy of the PK system antagonist, PC1, in contrasting neuropathic pain, neuroinflammation and immune activation in both sexes. Female control mice were characterized by high basal levels of (neuro) inflammatory markers that do not influence their pain thresholds. VCR induced in male mice a more marked mechanical allodynia and thermal hyperalgesia in comparison to females, who were precociously sensitive to cold stimuli. Considering neuroinflammation, VCR male mice showed a marked up-regulation of the chemokine PK2 and its receptors, cytokines and glial markers in both PNS and CNS. Differently, in VCR female mice, neuroinflammation is attenuated moving from PNS towards the spinal cord, the least affected station by VCR treatment. Chronic treatment with PC1 promptly reduced mechanical and thermal hypersensitivity in male mice, while in females, PC1 had a delayed and lower effect. In addition, PC1 almost always counteracted neuroinflammation in male mice, but its effect is not always evident in females. In conclusion, our data describes a sexual dimorphism in the pathophysiology of VCR-induced neuropathic pain and a different effect of the PK antagonist in the two sexes, confirming the importance of conducting experiments in both sexes.

1. Introduction

Vincristine (VCR), an alkaloid extracted from vinca, is commonly used, often in combination with other chemotherapeutic drugs, to treat a variety of cancers, such as acute lymphoblastic leukaemia, malignant lymphoma, neuroblastoma and other solid tumours [1]. However, VCR promotes a dose-dependent neurotoxicity, which is the main factor restricting its application [2,3]. Vincristine-induced neuropathy not only limits the dose of VCR and leads to treatment discontinuation but also significantly impacts patient physical and mental health [4,5]. Although the mechanisms underlying the development of neuropathy are not fully known, it was suggested a role of immune and glial cells that are recruited and/ or activated in the peripheral and central nervous system (PNS and CNS) after drug treatment [6,7]. Macrophages are highly sensitive to cytotoxic agents and their number is significantly increased in the sciatic nerve and dorsal root ganglia (DRGs) in rodent models of VCR-induced peripheral neuropathy (VIPN) [8–13]. Moreover, many studies have demonstrated that VCR induces the activation of microglia and astrocytes within the CNS, contributing to sustain a neuroinflammatory condition [8,12,14–16]. Activated immune and glial cells can in fact release inflammatory mediators such as cytokines and chemokines which foster neuroinflammation and sensitize neurons, contributing to painful neuropathy [17–19]. In this context, we previously demonstrated a role of a new family of chemokines, the

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prokineticins (PK), in the development of neuropathic pain of different origin [11,20,21] and, in particular, we showed that the activation of the PK system in the DRGs of VCR-treated male mice was important for sustaining an already ongoing neuroinflammatory process and promoting neuroinflammation in CNS [12]. In addition, the treatment with the PK system antagonist PC1 was able to counteract hypersensitivity to thermal and mechanical stimuli, preventing PK system activation and spinal cord neuroinflammation [12]. Many factors, including the cumulative dose, age, sex, ethnicity, and genetic variants of patients, might influence the development of VIPN [4,22]. In accordance, it is now recognized the presence of sex differences in the mechanisms underlying immune system activation and pain processing [23] and some clinical and preclinical studies are providing evidence on the presence of sex dimorphism in chemotherapy-induced peripheral neuropathy (CIPN) pathophysiology [24–26]. Sex could therefore be considered as a factor that may influence the development and magnitude of CIPN [27,28]. Most preclinical research on VIPN are performed using predominantly male mice; however, considering the possible implications of sex in the pain cascade, sex is a biological variable that must be considered. Here, for the first time, we describe in parallel the effect of VCR on neuropathic pain development in male and female mice in relation to neuroinflammation and PK system activation in the main stations involved in pain transmission from PNS to CNS. We also test the efficacy of the PK system antagonist, PC1, in contrasting neuropathic pain and neuroinflammation in both sexes.

2. Materials and methods

2.1. Animals

A total of 84 mice (Charles River Laboratories, Italy) were used in this study. In detail, 9-week-old male (n = 42) and female (n = 42) C57BL/6 J mice were housed 3 per cage (Macrolon type II cages, equipped with sawdust bedding, nesting and environmental enrichment), sex-separated, in standard conditions (i.e. 12 h of light/dark, temperature 22 ± 2 °C and humidity 55 ± 10 %) and with food and water *ad libitum*. After arrival at the animal facility, before starting the experiments, all mice were acclimatized for 7 days and subsequently accustomed to manipulation by daily handling (a few minutes/day), for a further 7 days. During their stay in the animal facility, mice underwent periodic veterinary checks. The ARRIVE guidelines and the 3 R principles were respected in all experiments. All procedures were conducted in accordance with the EU Directive 2010/63/EU and approved by the Animal Care and Use Committee of the Italian Ministry of Health (authorization number 709/2016 to SF).

2.2. Experimental protocol

The experimental schedule is shown in Fig. 1. Briefly, at day 0, six naïve mice of both sexes were sacrificed for subsequent biochemical analyses (baseline comparison). The other animals, males and females, were randomized (from the online source Graphpad - Randomly assign subjects to treatment groups) into two experimental groups: controls (CTR, n = 12 males; n = 12 females) and vincristine (VCR, n = 24 males; n = 24 females). The chemotherapeutic agent VCR (TOCRIS Bioscience™, USA) was injected intraperitoneally (i.p.) at a dose of 0.1 mg/kg every day for 14 consecutive days as previously described [12]. CTR mice were treated with saline solution (vehicle) (10 μl/g, i.p.). Seven days after the first injection of the chemotherapy drug, VCR mice of both sexes were further divided into two experimental groups (from the online source Graphpad). One group, VCR+PC1 (n = 12 males; n = 12 females), began the therapeutic treatment with the prokineticin receptor antagonist PC1 [29]. PC1 was administered subcutaneously (s.c.) at a dose of 150 μg/kg, twice a day for 7 consecutive days (from day 7 until the end of the VCR program (i.e. day 13)). The other VCR group (n = 12 males; n = 12 females) was treated with vehicle/ saline (10 μl/g, s.c.). All drugs were daily freshly prepared. Besides, to minimize possible interactions between the compounds (i.e. VCR and PC1), mice were treated with VCR always in the late afternoon, while the first administration of PC1 took place around 10 a.m. and the second approximately 5 h later (around 3 p.m.). Furthermore, in accordance with 3 R guidelines, in the present work CTR + PC1 group was not included because it has already been tested in our previous studies, observing that PC1 treatment in naïve mice did not alter either pain-like behaviors or biochemical parameters related to neuroinflammation [20,21,30].

2.3. Pain-like behavior evaluation

The von Frey test (mechanical allodynia), acetone drop test (thermal allodynia) and plantar test (thermal hyperalgesia) were used for pain-like behavior investigation. All tests were always carried out in the morning (between 7:00 – 10:00 a.m.) before any daily administration (VCR and/or PC1). Before each behavioral evaluation, the mice were familiarized with the new environment (behavioral room) for approximately 30 min and with the apparatus for approximately 15 min. The assessments were conducted before (day 0) and 3, 7, 10 and 14 days after the first VCR injection. Additionally, after the end of the VCR schedule (day 13) and of PC1 treatment, mice mechanical allodynia was monitored over time, once a week, until day 70. Furthermore, on day 7 (start of PC1 treatment) the acute effect of PC1 was evaluated by monitoring the responses to mechanical stimuli from 30 up to 270 min after its first administration. All assessments were performed in a blinded manner. For all tests, for each mouse, three measurements were

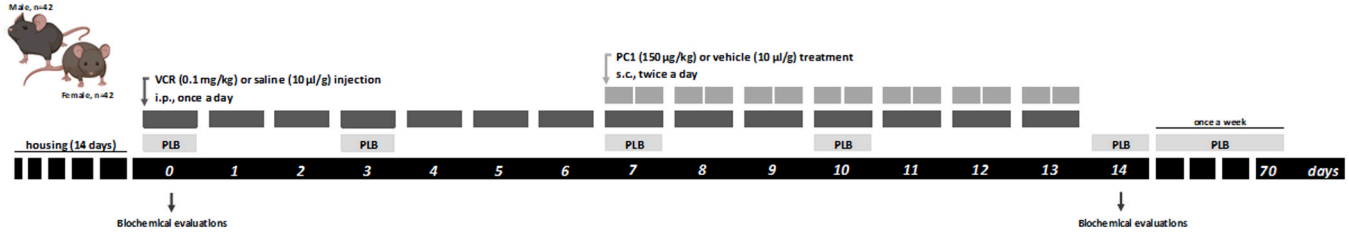

Fig. 1. Experimental protocol timeline. A total of 84 mice (males n = 42, females n = 42), 9-week-old and of the C57BL6J strain, were used in this study. After arrival in the animal facility, mice were acclimatized to the new environment and researchers for 2 weeks. At day 0, male and female naïve mice (n = 6/sex) were sacrificed. Moreover, before (day 0) and on days 3, 7, 10 and 14 of the experimental protocol, all mice were tested for pain-like behavior (PLB: mechanical and thermal allodynia and thermal hyperalgesia). At day 0, 48 mice (males n = 24, females n = 24) started the VCR schedule (VCR groups: 10 mg/kg, intraperitoneal (i.p.), once daily) while 24 mice (males n = 12, females n = 12) were treated with saline (CTR groups: 10 μg/g, i.p., once daily). At day 7, half of the VCR mice (males n = 12, females n = 12) began therapeutic treatment with PC1 (VCR+PC1 groups: 150 μg/kg, subcutaneous (s.c.), twice daily) while the other half of VCR animals (males n = 12, females n = 12) were treated with the vehicle (saline) (VCR groups: 10 μg/g, s.c., twice daily). At day 14, the end of the experimental protocol, 6 mice of each experimental group and sex were sacrificed for biochemical evaluations. The remaining animals (n = 6 for each experimental group and sex) were monitored over time for PLB evaluation parameters.

taken for each hind paw. The 3 values obtained from each hind paw were then averaged to obtain a single value/ paw. The two values (i.e. average of the right paw and average of the left paw) were subsequently averaged to obtain a single value for each mouse. Finally, the values thus obtained from mice of the same experimental group were averaged and used for statistical analysis.

- *Von frey test*: mechanical allodynia was assessed using the dynamic plantar aesthesiometer (Model: 37550 – Ugo Basile, Comerio, Italy). Briefly, the animals were placed in a modular plexiglass enclosure (1 mouse per module, dimensions of each module in mm: 96 ×96 x 140) positioned over a wire mesh. Through a blunt probe (Von Frey filament, Ø 0.5 mm) the mechanical stimulus was applied on the animal's hind paws mid-plantar surface, with increasing force (ranging up to 10 g in 10 s). When this stimulation becomes painful, the animal responds by removing the paw. The instrument displays on the monitor the force (in grams) at which the response occurred (e.g. withdrawal threshold, PWT) [11,12].
- *Acetone drop test*: thermal allodynia to cold stimuli was evaluated by placing an acetone drop (50 μl) in the middle of the plantar surface of the hind paws. Briefly, after drop application, the animal was placed inside a transparent plexiglass arena (in cm: 40 ×40 x h 50) and its behavior was monitored for 20 s (sec). If the animal showed no signs of withdrawing, beating or licking the paw under analysis, the observation ended (score equal to 0); on the contrary, if the animal responded to the stimulus, the observation was prolonged for another 20 sec, and using a point scale, scores were given as: 1, rapid withdrawal or single stroke of the paw; 2, prolonged withdrawal or repeated paw strikes; 3, repeated tapping and licking of the plantar surface of the paw [11].
- *Plantar test*: thermal hyperalgesia was tested using the Hargreaves apparatus (Model 37570 – Ugo Basile, Comerio, Italy). Briefly, the mice were placed in a modular plexiglass enclosure (1 mouse per module, dimensions of each module in mm: 96 ×96 x 140) positioned over an opaque glass plate. A constant thermal stimulus, generated by a focused light source (beam Ø 0.5 cm; intensity 20 I. R.), was applied through the glass panel to the plantar surface of mice hind paws. The response consists of the stimulated paw withdrawal. The measurement is recorded in seconds (paw withdrawal latency, PWL). Cut-off: 22 sec [12].

2.4. Tissue sampling and preparation

At day 0, for baseline characterization, a group of naïve males (n = 6) and females (n = 6) mice were sacrificed by decapitation. Furthermore, at the end of VCR/PC1 schedule, half of the mice in each experimental group (n = 6 male/group; n = 6 female/group) were killed by decapitation. The last administration of the drugs (VCR and/or PC1) was carried out the day before killing (day 13) and the tissues were collected the following morning (day 14). At the time of both sacrifices were collected:

i. blood from carotid artery blood; it was incubated for 3 h at room temperature and then for 2 h at 4°C, to allow correct coagulation without haemolysis. Subsequently, the blood was centrifuged at 21.000 g for 30 min at 4°C. Serum was removed and stored at -80°C for following PK2 evaluation by ELISA assay [11].
ii. peritoneal cells (PECs) for macrophage isolation as previously described [21]. Briefly, after mouse killing, the peritoneum was washed with 10 ml of RPMI 1640 medium (Sigma-Aldrich, Italy) supplemented with 10 % FBS (Gibco, Thermofisher Scientific, USA). An aliquot of the collected sample was used to check cell viability (exclusion test using trypan blue (Sigma-Aldrich, Italy)) and morphology of nuclei (using Turk's solution (Sigma-Aldrich, Italy)) for cell counting. PECs were dispensed into 24-well culture plates at a final concentration of 10^6/ml per well. Macrophage purification and isolation were performed by 2-h plate adhesion. Indeed, through two washes with PBS solution, the non-adherent cells were removed while the adherent cells (macrophage purity around 90 %) [21] were incubated with or without lipopolysaccharide (LPS, Sigma-Aldrich, Italy) to stimulate cytokine production. The stimulus was added to the macrophage culture in a final volume of 1 ml/well in complete RPMI (i.e., 1640 plus 10 % FCS, 1 % glutamine, 2 % streptomycin solution, and 0.2 -mercaptoethanol, 1 %) at a concentration of 1 μg/well. After 24 h of culture at 37°C and 5 % CO2, the supernatant was removed and adherent cells were detached from the plate by adding 500 μl/well of TRIzol™ Reagent (Invitrogen, USA) and stored at −80°C for subsequent evaluation of mRNA levels by RT-qPCR.
iii. nervous tissues largely involved in pain transmission, i.e. the sciatic nerve, DRGs (L4-L6) and spinal cord (L4-L6). Tissues were immediately frozen in liquid nitrogen and stored at −80°C until further use for evaluation of mRNA (RT-qPCR) levels of mediators involved in neuroinflammation [12].
iv. DRGs were homogenized in 150 μl of lysis buffer (ice-cold PBS + protease inhibitor cocktail, Roche Diagnostics, Italy). Samples were then centrifuged at 1000 g for 15 min at 4 °C and supernatant was stored at - 80°C for following PK2 (ELISA assay) and total protein content (Lowry's method) measurement [31].

2.5. ELISA

Specific mouse PK2 ELISA kit (Cusabio, USA) was used to measure the levels of PK2 in the serum and in DRG of CTR, VCR or VCR + PC1 male and female mice. Results are reported as ng/ml for serum as ng of PK2/mg total protein content for DRG or expressed as % vs. respective sex matched CTR [11].

2.6. RT-qPCR

The RNA was isolated from macrophages, sciatic nerve, DRGs (L4-L6) and spinal cord (L4-L6) using TRIzol™ reagent and subsequently reverse transcribed to cDNA with LunaScript® RT SuperMix Kit according to the respective manufacturers' instructions (Invitrogen, USA and BioLabs, UK, respectively). The expression levels of interest markers were analysed by Real Time PCR (RT-qPCR, QuantStudio 5™ - Thermofisher Scientific, USA) using Luna® Universal qPCR Master Mix (BioLabs, UK) and TaqMan probes (Thermofisher Scientific, USA) specific for the genes under study (i.e. PK2 - Mm01182450_g1, PKR1 - Mm00517546_m1, PKR2 - Mm00769571_m1, IL-1β - Mm00434228_m1; IL-6 -Mm00446190_m1; TNF-α - Mm00443258_m1, IL-10 - Mm00439616_m1, TLR4 - Mm00445274_m1, CD68 - Mm_03047343, CD11b - Mm00434455_m1, GFAP - Mm01253033_m1and ATF3 - Mm00476033_m1) according to the manufacturers' instructions. Each sample was run in triplicate alongside non-template controls (NTC). The mRNA levels of the studied genes were normalized to endogenous gene GAPDH (Mm99999915_g1) and expressed as $2^{-\Delta\Delta Ct}$ relative to the control group (specified in each figure legend).

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 (v3, San Diego, CA). Data analysis was run with parametric statistics being data normally distributed (as confirmed by Shapiro-Wilk test). For behavioral tests, data were analysed using Two-way ANOVA for repeated measures followed by Bonferroni's post-test. For biochemical evaluations, data were analysed with unpaired *t*-test (comparison of baseline values males vs. females) and with One-Way ANOVA followed by Bonferroni's post hoc test. Data represent mean ± SEM of 6 animals/group. For all analyses, differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Vincristine induced neuropathic pain in male and female mice. Effect of prokineticin antagonism treatment

In Fig. 2 it is reported the development of mechanical allodynia, thermal hyperalgesia and thermal allodynia in male and female mice after VCR treatment and the effect of PC1 administration. As shown in Fig. 2A and B, CTR male and female mice display similar basal withdrawal thresholds to both mechanical and thermal stimuli; however, after VCR treatment some differences are evident in the two sexes. Both male and female mice develop allodynia to mechanical stimuli (Fig. 2A), however the paw withdrawal thresholds to mechanical stimuli are lower in male mice at any measuring point (from day 3 until day 14). The response to thermal stimuli in female mice is delayed and always significantly less evident in comparison to that observed in male mice (Fig. 2B). A significant hyperalgesic response to thermal stimuli is already present in male mice after 3 days of VCR treatment remaining stable throughout the treatment while, in female mice, a significant decrease of paw withdrawal thresholds starts to be present after 7 days of VCR treatment and becomes more evident at the end of the treatment (day 14). In male mice PC1 significantly counteracts both mechanical allodynia and thermal hyperalgesia already after 3 days of chronic treatment (day 10), while in female mice its effect is delayed and becomes significant only at the end of the treatment (day 14). Fig. 2C shows that a clear thermal allodynia is evident in female VCR-treated mice starting from 7 days of VCR administration, while this condition is evident in male mice after 10 days of VCR treatment. PC1 is effective in contrasting thermal allodynia in both male and female mice; however, in male mice the effect is already present after 3 days of treatment (day 10) becoming higher after 7 days (day 14) while in female mice PC1 is effective only at the end of the treatment (day 14). In Fig. 2D it is shown the acute effect of PC1 on mechanical allodynia in male and female neuropathic mice. The effect of PC1 was evaluated after the first drug injection, after 7 days of VCR treatment, in the presence of a well-established hypersensitivity condition. In male mice, PC1 has a fast antiallodynic effect already evident 60 min after drug injection. After 120 min, we record the maximum effect of the drug which then begins to decrease and is no longer evident 210 min after its administration. In females, the effect of PC1 seems delayed with a maximum effect at 150 min after drug injection, which vanishes faster and is no longer evident at 210 min. Fig. 2E shows the effect of treatment discontinuation (VCR and PC1) on mechanical allodynia in male and female mice. In male mice, characterized by a more marked mechanical allodynia condition, hypersensitivity to innocuous mechanical stimuli is still evident after about 7 weeks since VCR treatment suspension, while in females, this effect is no longer evident after 5 weeks since discontinuation. The effect of PC1 is different in the two sexes in fact, in male, its effect is stronger and long-lasting as the antiallodynic effect of PC1 is still evident 5 weeks after drug discontinuation, while in females the small evident effect ends sooner.

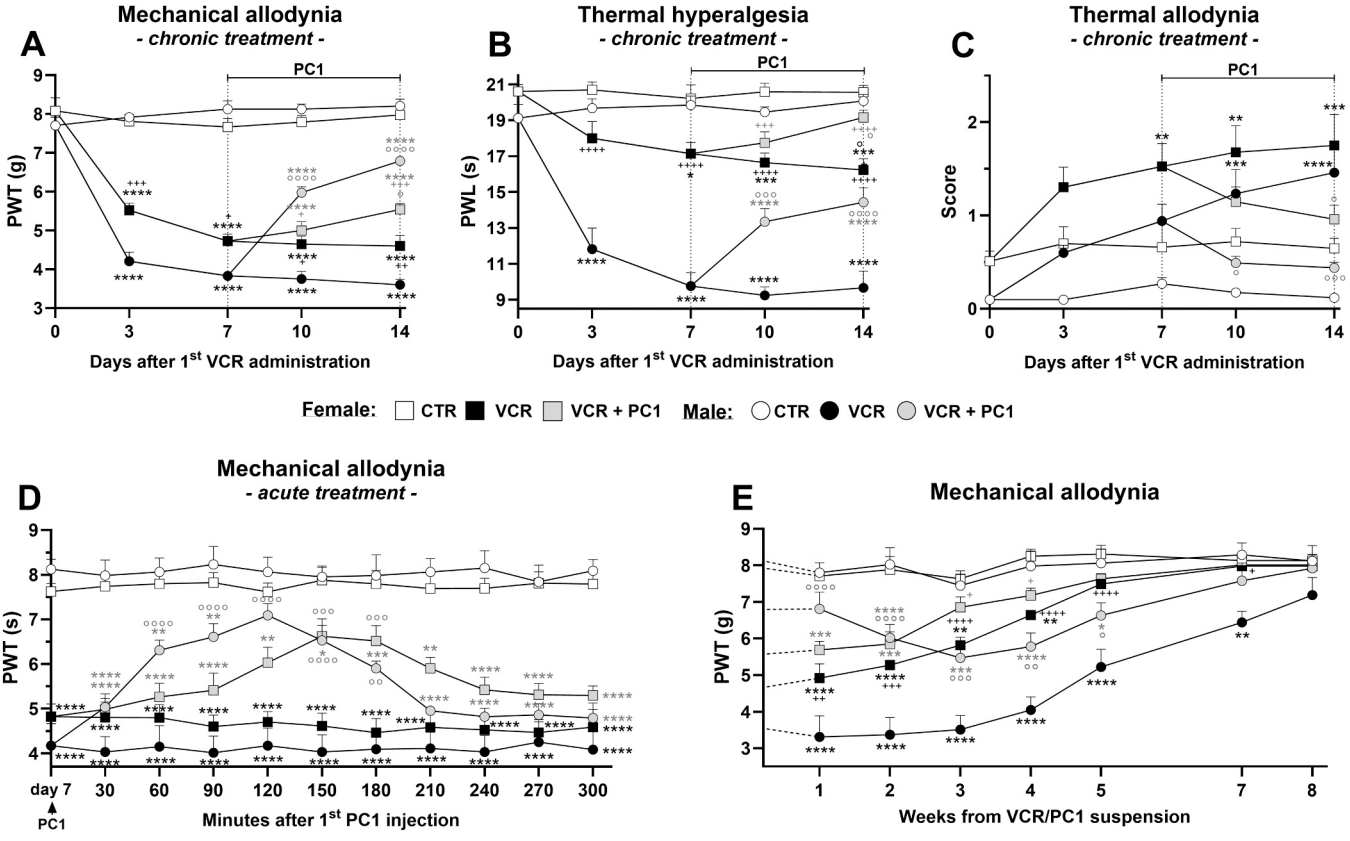

Fig. 2. VCR induced hypersensitivity in male and female mice, effect of PC1 antagonism. Effect of VCR treatment (0.1 mg/ kg i.p. once a day for 14 days) on mechanical allodynia (A), thermal hyperalgesia (B) and allodynia (C) development in male and female mice; the anti-allodynic and anti-hyperalgesic effect of PC1 was evaluated in both sexes after 3 and 7 days of chronic treatment (150 μg/ kg s.c., two times/ day; A-C) or after its first acute administration (D) performed after seven days of VCR treatment. (E) Effect of treatment discontinuation (VCR and PC1) on mechanical allodynia in male and female mice. Mice were discontinued from treatments starting from day 14. The presence of mechanical allodynia was monitored in the 8 following weeks from drug suspension. Data are mean ± SEM of 6 mice. Data were analysed using Two-way ANOVA for repeated measures followed by Bonferroni's post-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs sex matched CTR mice; °$p < 0.05$; °° $p < 0.01$; °°°$p < 0.001$; °°°° $p < 0.0001$ vs sex matched VCR mice; + $p < 0.05$; + + $p < 0.01$; + ++ $p < 0.001$; + +++ + $p < 0.0001$ vs male corresponding group.

3.2. Neuroinflammation in the sciatic nerve of male and female mice. Effect of VCR and prokineticin antagonism treatment

As shown in Fig. 3, female control mice are characterized by a physiological generalized enhanced expression of neuroinflammatory markers in the sciatic nerve if compared to control male mice (left graphs, Fig. 3A -N). In female mice, we detect higher levels of PK2, PKR1, proinflammatory cytokines, TLR4, Schwann cell (GFAP) and macrophage markers (CD68 and CD11B), simultaneously with a downregulation of the anti-inflammatory cytokine IL-10 in comparison to CTR male mice. At the end of VCR protocol, considering the nerve neuroinflammatory condition in both sexes, many differences emerged. In male VCR treated mice we detect an increase in the levels of the entire PK system: both PK2 (Fig. 3A) and PKRs (PKR1 and PKR2; Fig. 3B and C respectively) levels are upregulated while in female VCR mice we register an increase of PK2 only (Fig. 3A); PC1 is effective in reducing the upregulated PK system in both male and female mice. Also considering cytokines, male and female display a different response: in VCR male

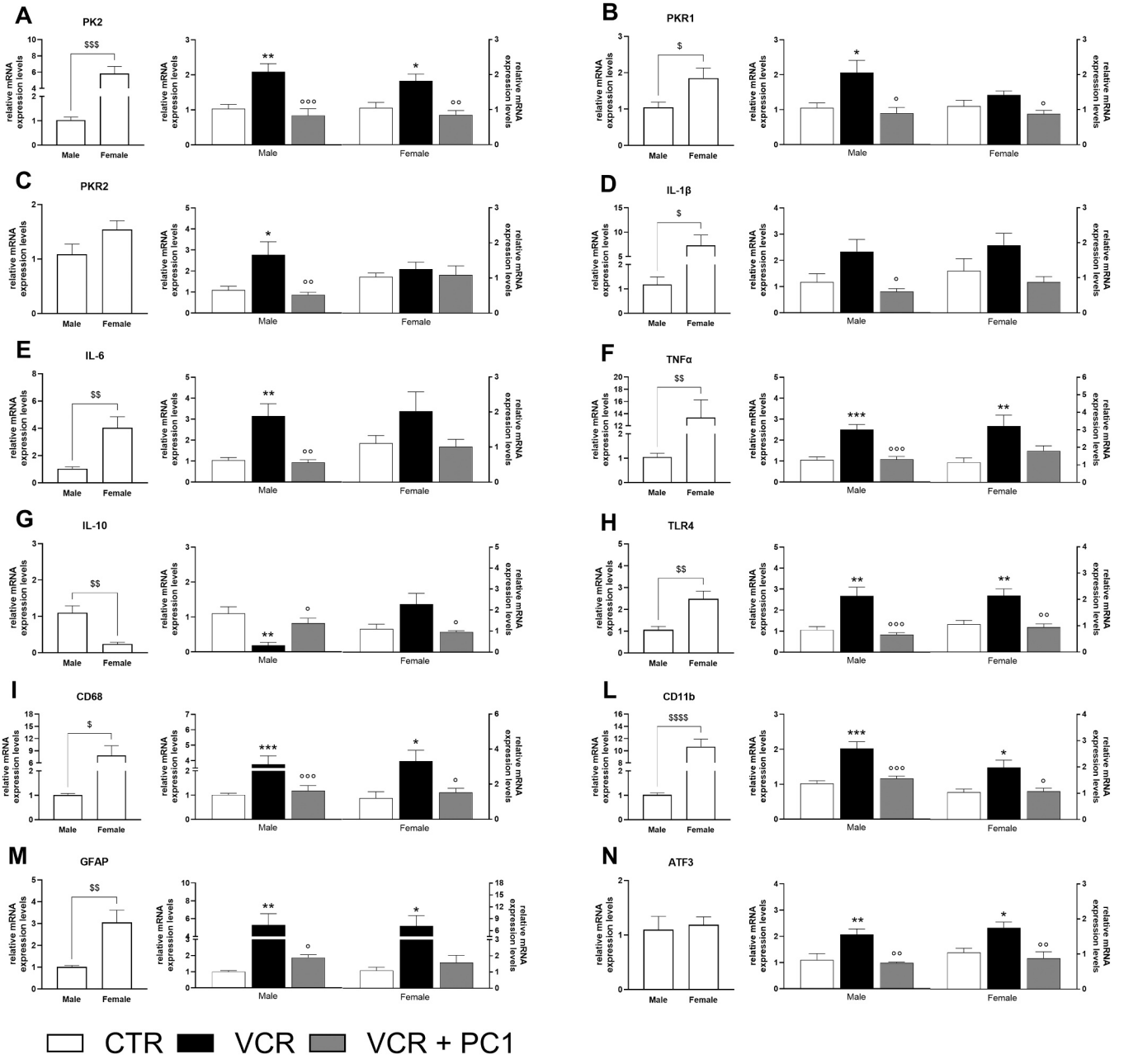

Fig. 3. Sciatic nerve neuroinflammation in male and female mice and effect of PC1 treatment. The left graph in each panel represents the mRNA levels of the neuroinflammatory markers in male and female CTR mice; results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over control male mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using unpaired t test. $ $p < 0.05$; $$ $p < 0.01$; $$$ $p < 0.001$ $$$$ $p < 0.0001$ vs CTR male mice. The right graph represents the mRNA levels of the neuroinflammatory markers measured in CTR, VCR (14 days of VCR treatment) and VCR+PC1 mice (14 days of VCR and 7 days of PC1 treatment). Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over respective male or female CTR mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post hoc test. Prokineticin system members (A, PK2; B, PKR1 and C, PKR2); pro/anti- inflammatory cytokines (D, IL-1β; E, IL-6; F, TNFα and G, IL-10), Schwann cells marker GFAP (H); macrophage markers CD68 (I) and CD11b (L), toll-like receptor TLR4 (M) and ATF3 (N). * $p < 0.05$, p < ** 0.01, *** 0.001 vs sex matched CTR mice; °$p < 0.05$, °°$p < 0.01$, °°° $p < 0.001$ vs sex matched VCR mice.

mice we observed high levels of all the proinflammatory cytokines: IL-1β (Fig. 3D), IL-6 (Fig. 3E) and TNFα (Fig. 3F) and a decrease of the anti-inflammatory cytokine IL-10 (Fig. 3G); PC1 was able to counteract this condition, reducing proinflammatory cytokines (Fig. 3D-3F) and enhancing the anti-inflammatory one (Fig. 3G). In females, we register a significant increase in the levels of TNFα (Fig. 3F) in the presence of a rising trend of IL-10 (Fig. 3G). PC1, in this case, was able to significantly reduce IL-10 and to partially reduce TNFα. We found a similar condition in male and female VCR-treated mice regarding to increased levels of TLR4 (Fig. 3H), of both macrophage markers CD68 and CD11b (Fig. 3I and L) and of Schwann cell marker GFAP (Fig. 3M). PC1 reduced TLR4 and the markers of activated macrophages in both sexes and it significantly counteract the increase of GFAP in male mice. In the nerve we also register an increase of the neuronal damage marker ATF 3 in both male and female VCR mice (Fig. 3N); PC1, reducing ATF3, exerts a neuroprotective effect in both sexes.

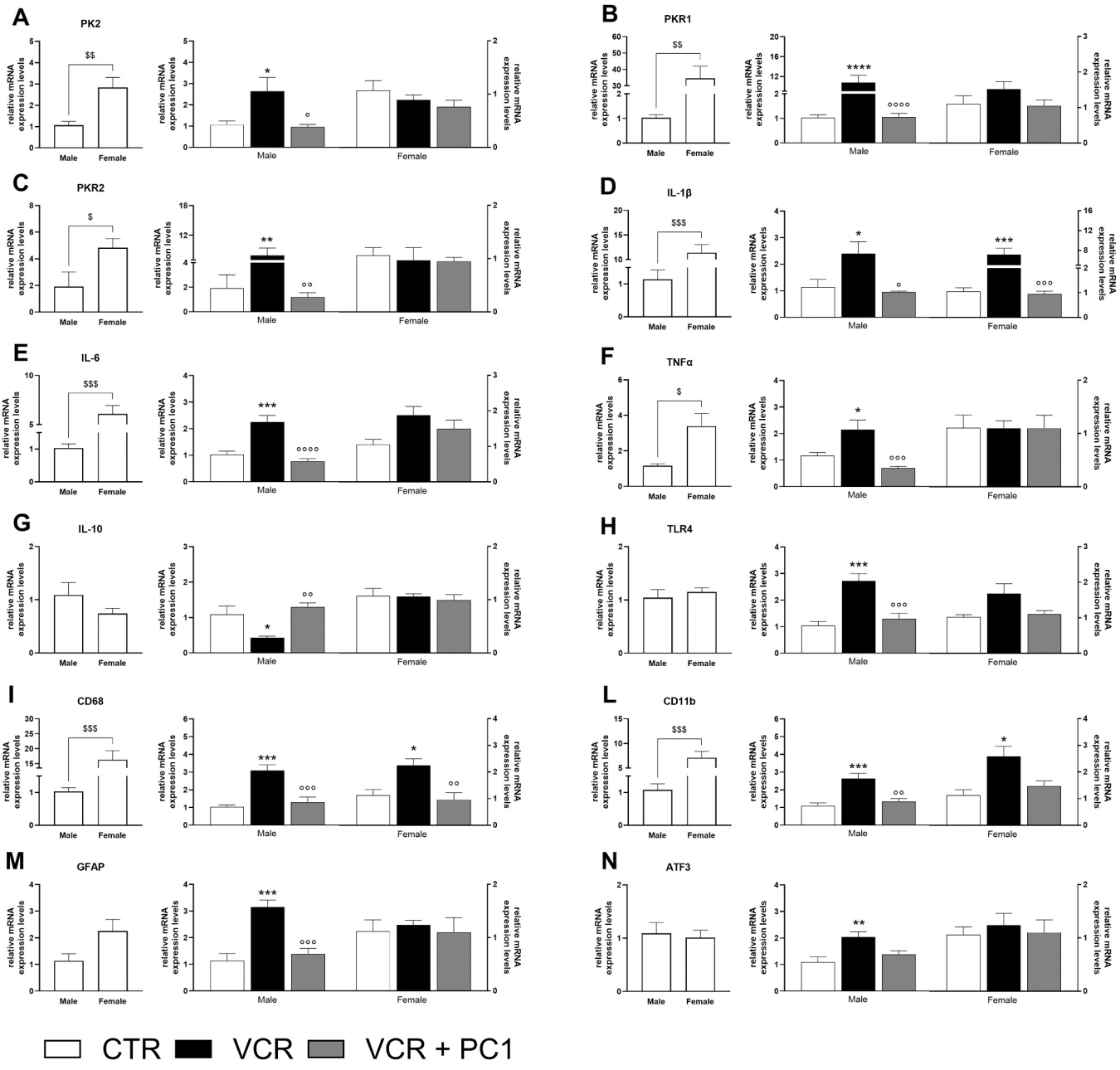

Fig. 4. DRG neuroinflammation in male and female mice and effect of PC1 treatment. The left graph in each panel represents the mRNA levels of the neuroinflammatory markers in male and female CTR mice. Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over control male mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using unpaired t test. $ $p < 0.05$; $$ $p < 0.01$; $$$ $p < 0.001$vs CTR male mice. The right graph represents the mRNA levels of the neuroinflammatory markers measured in CTR, VCR (14 days of VCR treatment) and VCR +PC1 mice (14 days of VCR and 7days of PC1 treatment). Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over respective male or female CTR mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post hoc test. Prokineticin system members (A-C, PK2, PKR1 and PKR2 respectively); pro/anti- inflammatory cytokines (D-G, IL-1β, IL-6, TNFα and IL-10 respectively); Schwann cells marker GFAP (H); macrophage markers CD68 (I) and CD11b (L); toll-like receptor TLR4 (M) and ATF3 (N). Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs sex matched CTR mice; °$p < 0.05$; °° $p < 0.01$; °°°$p < 0.001$; °°°° $p < 0.0001$ vs sex matched VCR mice.

3.3. Neuroinflammation in DRGs of male and female mice. Effect of VCR and prokineticin antagonism treatment

In the DRG, female control mice also show a clear enhancement in the expression of the neuroinflammatory markers in comparison to CTR male mice (left graph in the panels). This condition is characterized by elevated levels of the entire PK system (Fig. 4A-C), of proinflammatory cytokines (Fig. 4D-F) and macrophage markers (Fig. 4I and L). In the same figure (right graphs), it is possible to see that VCR treatment induces in male mice, but not in female ones, an upregulation of all PK system members, PK2 (Fig. 4A) and PKRs (Fig. 4B and C) and that PC1 counteracts the increase. PK2 mRNA alterations were also confirmed as proteins. As shown in Supplementary Fig. 1, VCR treatment also increases PK2 protein in male mice and PC1 can counteract the increase. On the other hand, no alterations in DRG PK2 protein content are evident of in female mice (experimental groups: CTR, VCR, VCR+PC1). Similarly, also cytokines appear significantly modulated mainly in male animals since in this sex we detect increased levels of the pro-inflammatory cytokines IL-1β (Fig. 4D), IL-6 (Fig. 4E) and TNFα (Fig. 4F), and a decrease of the anti-inflammatory cytokine IL-10

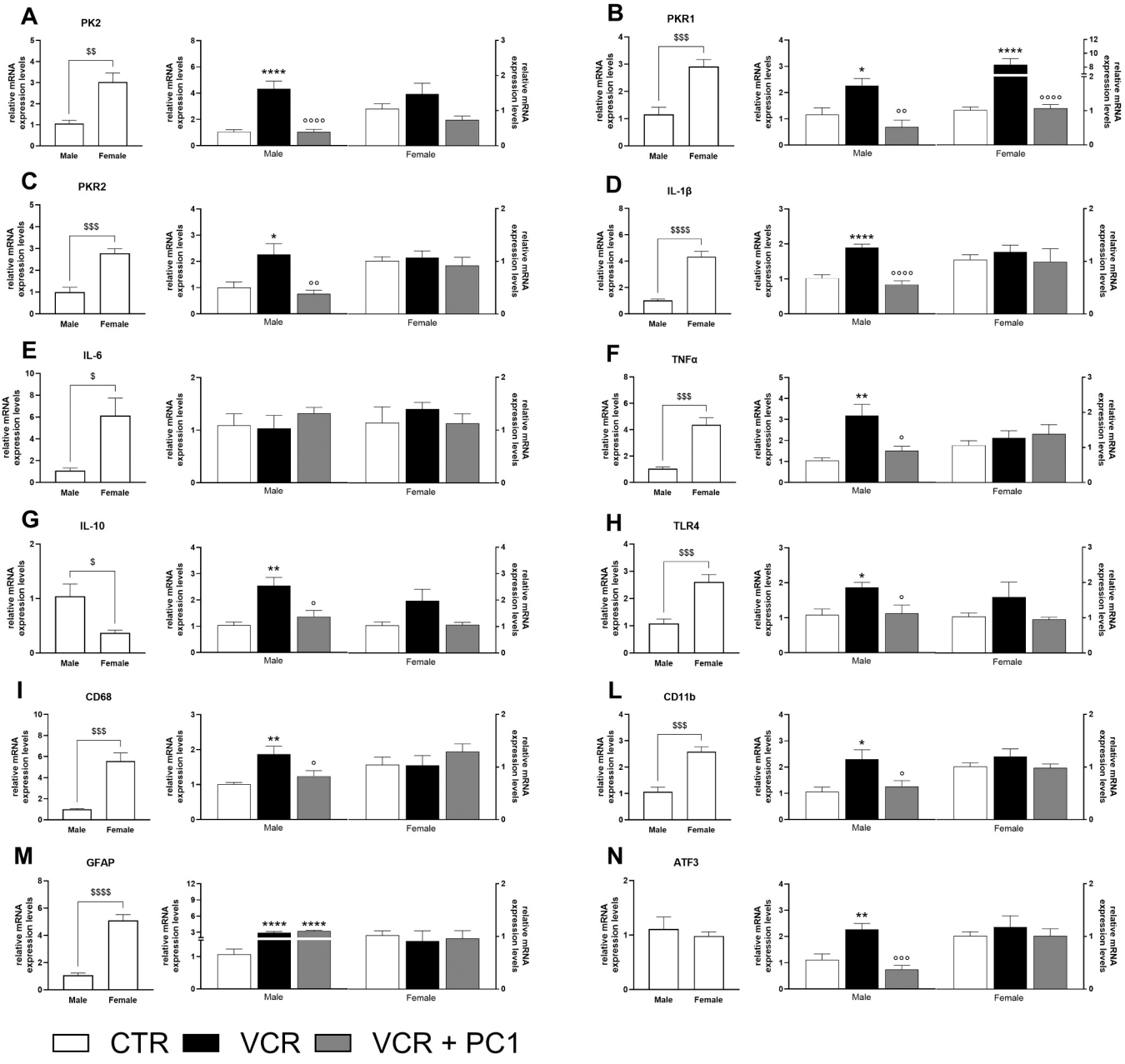

Fig. 5. Spinal cord neuroinflammation in male and female mice and effect of PC1 treatment. The left graph in each panel represents the mRNA levels of the neuroinflammatory markers in male and female CTR mice. Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over control male mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using unpaired t test. $ $p < 0.05$; $$ $p < 0.01$; $$$ $p < 0.001$; $$$$ $p < 0.0001$ vs CTR male mice. The right graph represents the mRNA levels of the neuroinflammatory markers measured in CTR, VCR (14 days of VCR treatment) and VCR +PC1 mice (14 days of VCR and 7 days of PC1 treatment). Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over respective male or female CTR mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post hoc test. Prokineticin system members (A-C, PK2, PKR1 and PKR2 respectively); pro/anti- inflammatory cytokines (D-G, IL-1β, IL-6, TNFα and IL-10 respectively); astrocyte marker GFAP (H); microglia markers CD68 (I) and CD11b (L); toll-like receptor TLR4 (M) and ATF3 (N). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ vs sex matched CTR mice; °$p < 0.05$; °° $p < 0.01$; °°°$p < 0.001$; °°°° $p < 0.0001$ vs sex matched VCR mice.

(Fig. 4G), while in female only IL-1β appears significantly upregulated in VCR mice (Fig. 4D). Both in male and in female mice PC1 restores altered cytokine levels (Fig. 4D-G). The neuroinflammatory condition, especially evident in male VCR mice, is also confirmed by the presence in this sex of increased TLR4 levels (Fig. 4H), upregulated levels of the activated macrophage markers CD68 and CD11b (Fig. 4I and L, respectively), satellite glial cell marker GFAP (Fig. 4M) and high levels of ATF3 (Fig. 4N). PC1 treatment significantly reduces TLR4 (Fig. 4H), macrophage markers increase (Fig. 4I and L) and GFAP (Fig. 4M). In female VCR-treated mice we only detect a significant increase of both CD68 and CD11b (Fig. 4I and L) and PC1 significantly reduces CD68 (Fig. 4I).

3.4. Neuroinflammation in the spinal cord of male and female mice. Effect of VCR and prokineticin antagonism treatment

The general increase in the expression of neuroinflammatory markers observed in the peripheral nervous system stations of female control mice is also evident in the spinal cord (left graphs in panels). In fact, also in the spinal cord, female control mice display increased levels of PK system members (PK2 and PKRs, Fig. 5A-C), increased proinflammatory cytokines (Fig. 5D-F), TLR4 (Fig. 5H), glial markers (CD68, CD11b and GFAP; Fig. 5I-5M) and decreased IL-10 levels (Fig. 5G) in comparison to control male mice. In the right graphs of the same panels, it is possible to observe that VCR treatment was practically unable to induce any alteration in the spinal cord of female mice where we only register a significant increase of PKR1 that is contrasted by PC1 (Fig. 5B). Differently from female mice, also at spinal cord level, VCR treated male mice are characterized by a clear neuroinflammatory condition with increased levels of PK system members (Fig. 5A-5C), cytokines (Fig. 5D, F and G), TLR4 (Fig. 5H), glial markers (Fig. 5I-M) and ATF3 (Fig. 5N). PC1 treatment was able to counteract this neuroinflammatory condition without showing a full effect on GFAP (Fig. 5M).

3.5. PK2 content in the serum of male and female mice. Effect of VCR and prokineticin antagonism treatment

In Fig. 6 it is reported the PK2 serum quantity. PK2 serum levels are similar in control male and female mice (left graph); as shown in the right graph, VCR treatment induces an increase of PK2 serum levels in both male and female mice, while PC1 treatment reduces them, maintaining the levels low.

3.6. PK system and cytokines expression in macrophages of male and female mice. Effect of VCR and PK antagonism treatment

As shown in Fig. 7, also in peripheral macrophages, female control mice are characterized by higher basal mRNA levels of inflammatory markers (PK2, PKRs, IL-6 and IL-1β) in comparison to control male mice (left graph for each panel). VCR treatment induces in both sexes (right graphs), an increase of PK2 and PKR1 (Fig. 7A and B, respectively) as well as of the proinflammatory cytokines IL-1β (Fig. 7D) and IL-6 (Fig. 7E). The VCR effect is counteracted by PC1 treatment.

4. Discussion

Nowadays it is established that sex represents an important biological variable that may influence the development of chemotherapy-induced peripheral neuropathy-CIPN [4,26]. There are several sex specific differences in the mechanisms underlying immune system activation and pain processing [23]. It is suggested, for example, that microglia, the principal immune cell of the CNS, contributes to pain hypersensitivity in male but not in female experimental models of neuropathic pain [23,32]. In neuropathic pain conditions, despite similar microglia proliferation in the spinal cord dorsal horn in both sexes, females do not upregulate P2X4Rs and use a microglia-independent pathway to mediate pain hypersensitivity [33,34]. Moreover, Toll-like receptor 4 (TLR4), specifically expressed on microglia in the CNS, contributes to pain hypersensitivity in male mice only [35]. These profound sex differences highlight the importance of including subjects of both sexes in preclinical pain research to uncover the role of sex in the pathophysiology of CIPN and to improve the translational relevance of the preclinical pain research.

In our study, in a vincristine-induced neuropathy model, we use in parallel both male and female mice and we observed in the two cohorts the development of hypersensitivity and neuroinflammation in the main stations involved in pain signaling. Our data suggest that male and female mice display different responses to mechanical and thermal stimuli. In particular, male mice treated with VCR are always characterized by more marked mechanical allodynia as well as thermal hyperalgesia compared to female VCR-treated mice; in contrast, female mice seem precociously sensitive to cold stimuli. A sex-dependent response to vinka alkaloids was also described in rats after chronic vinblastine (VBL) exposure. In this case, male rats were more susceptible to motor impairments induced by VBL in comparison to female rats [36]. A sexual dimorphism was also described for other antitumoral agents, for example in paclitaxel (PTX) induced neuropathy; PTX-induced cold allodynia was more robust in female mice [37] and a greater mechanical hypersensitivity was found in males treated with PTX [38] when compared to female mice. About the impact of sex in the chemotherapy-induced neuropathic pain provoked by cisplatin (CIS), contradictory results exist. Some studies revealed that the mechanical and/or cold allodynia caused by CIS are similarly displayed in male and female animals [37,39], whereas another showed sex discrepancies in the maintenance of mechanical allodynia [40]. In order to understand

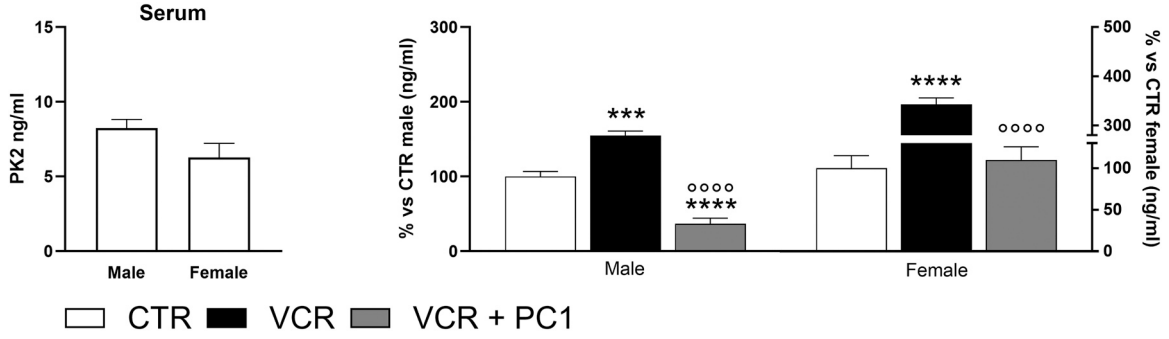

Fig. 6. Serum levels of PK2 measured by ELISA after 14 days of chronic VCR treatment (day 14 corresponding to 7 days of chronic PC1 administration). The left graph in the panel represents the levels of PK2 (ng/ ml) measured in CTR male and female mice (day 14). Right graph, serum levels of PK2 expressed as % vs respective sex matched CTR. Data are mean ± SEM of 6 mice. Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post hoc test. *** $p < 0.001$; **** $p < 0.0001$ vs sex matched CTR mice; °°°° $p < 0.0001$ vs sex matched VCR mice.

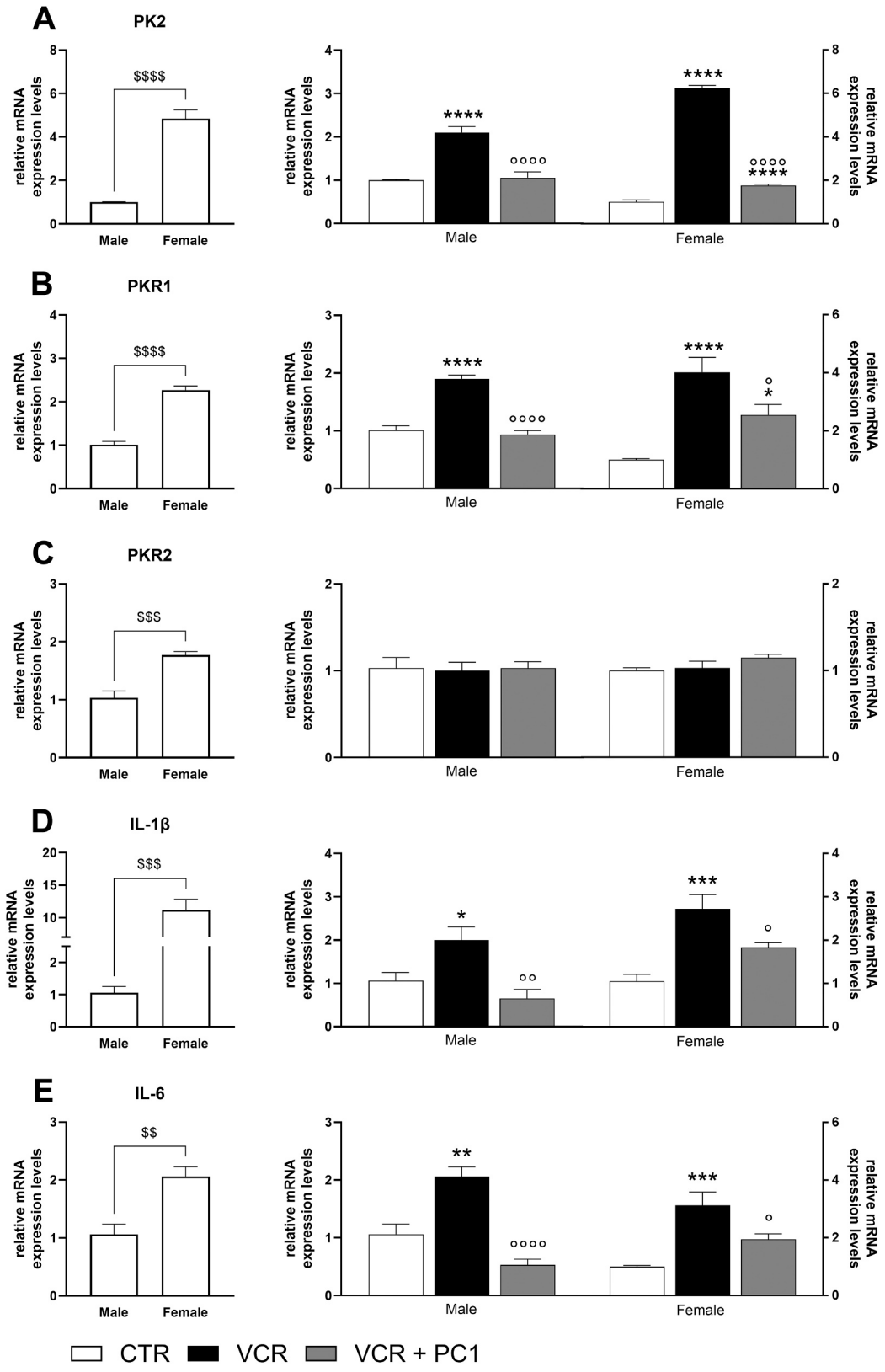

Fig. 7. Peritoneal macrophage mRNA levels of PK system members and proinflammatory cytokines in male and female mice. The left graph in each panel represents the mRNA levels of PK2 (A), PKR1 (B), PKR2 (C), IL-1β (D) and IL-6 (E) measured in male and female CTR mice. Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over control male mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using unpaired t test. $$ $p < 0.01$; $$$ $p < 0.001$; $$$$ $p < 0.0001$ vs CTR male mice. The right graph represents the mRNA levels of the same markers measured in CTR, VCR (14 days of VCR treatment) and VCR +PC1 mice (14 days of VCR and 7days of PC1 treatment). Results are normalized on the housekeeping gene GAPDH and expressed as fold of changes over respective male or female CTR mice. Data are mean ± SEM of 6 mice. Statistical analysis was performed using One Way ANOVA followed by Bonferroni's post hoc test. * $p < 0.05$;** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs sex matched CTR mice; ° $p < 0.05$; °°°° $p < 0.0001$ vs sex matched VCR mice.

the different development of pain symptomatology in males and females after VCR, we investigated the neuroinflammatory status in the two sexes. Our data show that CTR female mice are characterized by high basal levels of neuroinflammatory markers in the main stations of pain signaling, indeed, in the sciatic nerve, DRGs and spinal cord of CTR female mice we registered a significant up regulation of PK system members, of proinflammatory cytokines, of glial markers and a down regulation of the anti-inflammatory cytokine IL-10 in comparison to male mice. This condition, however, does not significantly affect mice thresholds to mechanical and thermal stimuli; in fact, male and female control mice display similar response thresholds. When male and female mice were treated for 14 consecutive days with VCR, the situation changes: we register differences in neuroinflammation between male and female but, in this case, we have a clear and more marked neuroinflammatory condition in male mice. PK2 and PKRs mRNA levels are increased in all nervous stations (sciatic nerve, DRGs, spinal cord) only in male mice and, in the same animals, also the mRNA for TLR4, proinflammatory cytokines, microglial/ activated macrophages markers, Schwann/ satellite/ astrocyte marker and the neuronal damage marker ATF3 is almost always upregulated with a few exceptions. This condition, however, does not occur in females where the main station affected by VCR treatment is the peripheral nerve in which we observe a similar condition to that observed in males. Our results show that, in female mice, VCR-induced neuroinflammation seems attenuated as we move from the periphery towards the spinal cord, which is the station less affected by VCR treatment in this sex. We could hypothesize that the elevated physiological levels of neuroinflammatory markers in pain transmission stations in female mice might contribute to the diminished PNS neuroinflammation and the subsequent absence of spinal cord alterations observed after VCR insult. As above described, microglia and TLR4 expressed by it are important for pain hypersensitivity in male mice [35,41] and our data described, in accordance, an increase of these markers in the DRG and spinal cord of male mice but not in female mice thus resulting in a less marked mechanical allodynia and thermal hyperalgesia in female VCR mice. Consistently, Iguchi and colleagues [42] described, following VCR treatment, a robust macrophage expansion in the lumbar DRGs of male mice that contributes to mechanical allodynia. The authors also showed a male-specific trend of upregulation of IL-1β, IL-6 and TNFα in the DRG following VCR exposure suggesting that VCR induced inflammatory responses at least partly through the P2x4-P2x7 pathway in the Ls-DRG in male mice [42]. In addition, other preclinical studies revealed that the activation of p38 MAPK in the microglia cells of the spinal cord plays a role in neuropathic pain, resulting in an increased release of pro-inflammatory cytokines, such as TNF-α and IL-1β. In accordance, the inhibition of p38 MAPK has been shown to effectively reverse mechanical allodynia and reduce pain in various models of neuropathic pain [43,44]. Our data clearly shows that in female mice neither CD11b nor CD68, markers for microglia and activated macrophages, are upregulated in the spinal cord and, at the same time, no other alterations (cytokines, PK system, astrocytic marker, TLR4 and ATF3) are detected in the spinal cord. Differently, in the spinal cord of VCR male mice we detected a clear neuroinflammatory condition with an upregulation of all glial markers, of the main cytokines, of the entire PK system, of TLR4 and of the marker for neuronal damage ATF3. We previously demonstrated that the activation of the PK system in DRGs is important to sustain and amplify an ongoing neuroinflammatory loop already evident after 7 days of VCR treatment and that the upregulation of the PK system is an important step for activating the spinal cord neuroinflammatory pathway that is fundamental for pain chronicization [12]. Given the established role of PK2 upregulation in driving spinal cord neuroinflammation in CIPN [11,12], the absence of PK2 upregulation (both mRNA and protein) at the DRG level in female mice might suggest the lack of observed spinal cord alterations. The attenuated neuroinflammatory response in female VCR-treated mice could explain their reduced mechanical allodynia and thermal hyperalgesia. While this is a plausible hypothesis, further experiments are needed to confirm it. Our data also describes a clear correspondence between PK2 mRNA and protein levels in DRG suggesting that transcriptional control is the dominant factor driving changes in protein content and associated biological function. However, our data show that female VCR mice seem to be more precociously sensible to cold stimuli than male mice. The unmyelinated C-fibres and the slightly myelinated Aδ-fibres are involved in the perception of non-noxious and noxious cold stimuli [45,46]. Recent studies have elucidated multiple mechanisms underlying the onset and expression of cold allodynia and hyperalgesia including an upregulation of glial cell-derived neurotrophic factor (GDNF) family receptor alpha-2 (GFRα3) signalling [47], upregulation of transient receptor potential cation channel subfamily M member 8 (TRPM8), dysfunction of sodium channels, increased sensitization of TRPA1 channels, dysfunction of calcium channels and potassium hyperpolarization-activated cation channels, disinhibition of C-polymodal nociceptive fibres with concurrent loss of Aδ fibres [48–50]. It could be possible, but it should be verified, that one of these alterations might explain the cold allodynia time course in female mice. In this context, it was also suggested an influence of sex hormones on TRP channel function and their signalling pathways that could influence sex differences in CIPN [51]. For example, it was described that oestrogens can promote TRPV1 signalling [52] and despite its insensitivity to cold, TRPV1 can enhance membrane potential changes and electrical firing of TRPM8 + neurons in response to cold stimulation. In contrast, progesterone seems to have a protective role in CIPN development [53], in fact it was described the capacity of progesterone to prevent neuropathy and to exert an antinociceptive action in VCR-treated rats [54,55]. These effects are due to a progesterone-driven downregulation of TRPV1, TRPA1 and TRPV4 channels [56,57]. The protective effect of sex hormones might also explain the fast recovery after VCR discontinuation observed in female mice but it should be investigated. The potential involvement of hormones warrants more extensive investigation in future studies. In our experiments, drug treatments were initiated independently of the estrous cycle phase. While this approach is clinically relevant, the inability to directly correlate observed variations with the cycle phase could represent a limitation.

Allodynia to mechanical stimuli was no more evident in female mice that underwent VCR treatment after about 30 days of treatment suspension, while this condition is still evident in male mice also after 50 days. The chronic treatment with the PK system antagonist PC1 promptly reduces hypersensitivity to mechanical and thermal stimuli in male mice while, in females, PC1 seems to take a little longer to act on both allodynia and hyperalgesia also showing lower efficacy. In accordance, our data show that PC1 can almost always counteract the neuroinflammatory condition in the main stations involved in pain pathways in male VCR-treated mice while, in females, it seems less effective as it fails to counteract to GFAP and TNFα and CD11b upregulation in the PNS. Although, PC1 can reduce PK2 levels in PNS in both male and female mice, likely blocking the direct sensitization effect of PK2 on nociceptors, the differences observed in the two sexes might be due to a lower efficacy of PC1 to reduce neuroinflammation in PNS in female mice. Further investigations are warranted to elucidate the mechanisms underlying the observed sex differences. Emerging research highlights that males and females frequently utilize distinct cell pathways to mediate pain and neuroinflammation, which can consequently alter the efficacy of targeted pharmacological treatments. As we already described for bortezomib-induced neuropathy [11], here we found high PK2 levels in the serum of both male and female VCR-treated neuropathic mice. The damage exerted by VCR in PNS stations is important for inducing the recruitment and activation of peripheral immune cells that contribute to sustaining a neuroinflammatory condition in PNS stations. Peripheral leukocytes are probably the main source of the chemokine, since monocytes, granulocytes, and lymphocytes produce and release PK2 when activated [30,58,59]. Moreover, our data show that macrophages isolated from both VCR-treated male and female mice express

high levels of both PK2 and PKR1 receptor in the presence of elevated levels of proinflammatory cytokines. Our results clearly show that PC1 counteracts peripheral neuroinflammation and reduces the activation of peripheral immune cells and likely the consequent release of systemic PK2.

5. Conclusion

Our study suggests a sexual dimorphism in the pathophysiology of vincristine induced neuropathic pain but also, for the first time, a sex dependent response to PC1 treatment. The effect of PC1 on pain, although evident in both sexes, seems delayed in female mice and its effect in contrasting neuroinflammation seems more efficacious in male mice. The data confirms the importance of conducting experiments in both sexes to provide insightful information for clinically efficacious interventions.

Author contributions

SF and PS conceptualization. GA and SF performed the experiments. GA, GG and SF data analysis and graph. SF writing original draft, VO synthesis PC1. PS, RL and ATB critically revised the manuscript. All the authors approved and reviewed the final manuscript.

CRediT authorship contribution statement

Valentina Onnis: Writing – review & editing, Validation. **Paola Sacerdote:** Writing – review & editing, Supervision, Conceptualization. **Silvia Franchi:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Giada Amodeo:** Writing – review & editing, Software, Methodology, Investigation, Data curation. **Giulia Galimberti:** Writing – review & editing, Software, Data curation. **Roberta Lattanzi:** Writing – review & editing. **Anna Teresa Brini:** Writing – review & editing, Funding acquisition.

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Declaration of Competing Interest

The authors declare no competing interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118596.

Data availability

Data will be made available on request.

References

[1] J. Škubník, V.S. Pavlíčková, T. Ruml, S. Rimpelová, Vincristine in combination therapy of cancer: emerging trends in clinics, Biol 10 (9) (2021) 849, https://doi.org/10.3390/biology10090849.
[2] A.A. Argyriou, J. Bruna, P. Marmiroli, G. Cavaletti, Chemotherapy-induced peripheral neurotoxicity (CIPN): an update, Crit. Rev. Oncol. /Hematol. 82 (1) (2012) 51–77, https://doi.org/10.1016/j.critrevonc.2011.04.012.
[3] G.Z. Li, Y.H. Hu, D.Y. Li, Y. Zhang, H.L. Guo, Y.M. Li, F. Chen, J. Xu, Vincristine-induced peripheral neuropathy: a mini-review, Neurotoxicology 81 (2020) 161–171, https://doi.org/10.1016/j.neuro.2020.10.004.
[4] M. Seretny, G.L. Currie, E.S. Sena, S. Ramnarine, R. Grant, M.R. MacLeod, L. A. Colvin, M. Fallon, Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis, Pain 155 (12) (2014) 2461–2470, https://doi.org/10.1016/j.pain.2014.09.020.
[5] J.C. Santos, L.M. Pyter, Neuroimmunology of behavioral comorbidities associated with cancer and cancer treatments, Front. Immunol. 9 (2018) 1195, https://doi.org/10.3389/fimmu.2018.01195.
[6] L.A. Colvin, Chemotherapy-induced peripheral neuropathy: where are we now? Pain 160 (1) (2019) S1–S10, https://doi.org/10.1097/j.pain.0000000000001540.
[7] G. Fumagalli, L. Monza, G. Cavaletti, R. Rigolio, C. Meregalli, Neuroinflammatory process involved in different preclinical models of Chemotherapy-Induced peripheral neuropathy, Front. Immunol. 11 (2021) 626687, https://doi.org/10.3389/fimmu.2020.626687.
[8] N. Kiguchi, T. Maeda, Y. Kobayashi, S. Kishioka, Up-regulation of tumor necrosis factor-alpha in spinal cord contributes to vincristine-induced mechanical allodynia in mice, Neurosci. Lett. 445 (2) (2008) 140–143, https://doi.org/10.1016/j.neulet.2008.09.009.
[9] K. Montague, M. Malcangio, The therapeutic potential of Monocyte/Macrophage manipulation in the treatment of Chemotherapy-Induced painful neuropathy, Front. Mol. Neurosci. 10 (2017) 397, https://doi.org/10.3389/fnmol.2017.00397.
[10] K. Montague, R. Simeoli, J. Valente, M. Malcangio, A novel interaction between CX3CR1 and CCR2 signalling in monocytes constitutes an underlying mechanism for persistent vincristine-induced pain, J. Neuroinflamm. 15 (1) (2018) 101, https://doi.org/10.1186/s12974-018-1116-6.
[11] G. Moschetti, G. Amodeo, D. Maftei, R. Lattanzi, P. Procacci, P. Sartori, G. Balboni, V. Onnis, V. Conte, A. Panerai, P. Sacerdote, S. Franchi, Targeting prokineticin system counteracts hypersensitivity, neuroinflammation, and tissue damage in a mouse model of bortezomib-induced peripheral neuropathy, J. Neuroinflamm. 16 (1) (2019) 89, https://doi.org/10.1186/s12974-019-1461-0.
[12] G. Moschetti, G. Amodeo, M.S. Paladini, R. Molteni, G. Balboni, A. Panerai, P. Sacerdote, S. Franchi, Prokineticin 2 promotes and sustains neuroinflammation in vincristine treated mice: focus on pain and emotional like behavior, Brain Behav. Immun. 82 (2019) 422–431, https://doi.org/10.1016/j.bbi.2019.09.012.
[13] X. Yu, H. Liu, K.A. Hamel, M.G. Morvan, S. Yu, J. Leff, Z. Guan, J.M. Braz, A. I. Basbaum, Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain, Nat. Commun. 11 (1) (2020) 264, https://doi.org/10.1038/s41467-019-13839-2.
[14] Y. Shen, Z.J. Zhang, M.D. Zhu, B.C. Jiang, T. Yang, Y.J. Gao, Exogenous induction of HO-1 alleviates vincristine-induced neuropathic pain by reducing spinal glial activation in mice, Neurobiol. Dis. 79 (2015) 100–110, https://doi.org/10.1016/j.nbd.2015.04.012.
[15] L. Zhou, Y. Hu, C. Li, Y. Yan, L. Ao, B. Yu, W. Fang, J. Liu, Y. Li, Levo-corydalmine alleviates vincristine-induced neuropathic pain in mice by inhibiting an NF-kappa B-dependent CXCL1/CXCR2 signaling pathway, Neuropharmacology 135 (2018) 34–47, https://doi.org/10.1016/j.neuropharm.2018.03.004.
[16] E.S. El-Sawaf, N.N. El Maraghy, H.S. El-Abhar, H.F. Zaki, B.N. Zordoky, K. A. Ahmed, N. Abouquerin, A.F. Mohamed, Melatonin mitigates vincristine-induced peripheral neuropathy by inhibiting TNF-α/astrocytes/microglial cells activation in the spinal cord of rats, while preserving vincristine's chemotherapeutic efficacy in lymphoma cells, Toxicol. Appl. Pharm. 492 (2024) 117134, https://doi.org/10.1016/j.taap.2024.117134.
[17] N. Kiguchi, T. Maeda, Y. Kobayashi, F. Saika, S. Kishioka, Involvement of inflammatory mediators in neuropathic pain caused by vincristine, Int. Rev. Neurobiol. 85 (2009) 179–190, https://doi.org/10.1016/S0074-7742(09)85014-9.
[18] X.T. Ji, N.S. Qian, T. Zhang, J.M. Li, X.K. Li, P. Wang, D.S. Zhao, G. Huang, L. Zhang, Z. Fei, D. Jia, L. Niu, Spinal astrocytic activation contributes to mechanical allodynia in a rat chemotherapy-induced neuropathic pain model, PloS One 8 (4) (2013) e60733, https://doi.org/10.1371/journal.pone.0060733.
[19] L. Zhou, L. Ao, Y. Yan, W. Li, A. Ye, Y. Hu, W. Fang, Y. Li, The therapeutic potential of chemokines in the treatment of Chemotherapy- induced peripheral neuropathy, Curr. Drug Targets 21 (3) (2020) 288–301, https://doi.org/10.2174/1389450120666190906153652.
[20] D. Maftei, V. Marconi, F. Florenzano, L.A. Giancotti, M. Castelli, S. Moretti, E. Borsani, L.F. Rodella, G. Balboni, L. Luongo, S. Maione, P. Sacerdote, L. Negri, R. Lattanzi, Controlling the activation of the Bv8/prokineticin system reduces neuroinflammation and abolishes thermal and tactile hyperalgesia in neuropathic animals, Br. J. Pharm. 171 (21) (2014) 4850–4865, https://doi.org/10.1111/bph.12793.
[21] M. Castelli, G. Amodeo, L. Negri, R. Lattanzi, D. Maftei, C. Gotti, F. Pistillo, V. Onnis, C. Congu, A.E. Panerai, P. Sacerdote, S. Franchi, Antagonism of the prokineticin system prevents and reverses allodynia and inflammation in a mouse model of diabetes, PloS One 11 (1) (2016) e0146259, https://doi.org/10.1371/journal.pone.0146259.
[22] D.L. Hershman, C. Till, J.D. Wright, J.D. Awad, S.D. Ramsey, W.E. Barlow, L. M. Minasian, J. Unger, Comorbidities and risk of Chemotherapy-Induced peripheral neuropathy among participants 65 years or older in southwest oncology group clinical trials, Am. J. Clin. Oncol. 34 (25) (2016) 3014–3022, https://doi.org/10.1200/JCO.2015.66.2346, 155 (2014), pp. 2461-2470.
[23] T.N. Friedman, O. La Caprara, C. Zhang, K. Lee, J. May, C.A. Faig, T. Baldwin, J. R. Plemel, A.M.W. Taylor, B.J. Kerr, Sex differences in peripheral immune cell activation: implications for pain and pain resolution, Brain Behav. Immun. 114 (2023) 80–93, https://doi.org/10.1016/j.bbi.2023.07.029.
[24] M. Davidson, A.D. Wagner, K. Kouvelakis, H. Nanji, N. Starling, I. Chau, D. Watkins, S. Rao, C. Peckitt, D. Cunningham, Influence of sex on chemotherapy efficacy and toxicity in oesophagogastric cancer: a pooled analysis of four

randomised trials, Eur. J. Cancer 121 (2019) 40–47, https://doi.org/10.1016/j.ejca.2019.08.010.
[25] A.D. Wagner, Sex differences in cancer chemotherapy effects, and why we need to reconsider BSA-based dosing of chemotherapy, ESMO Open 5 (5) (2020) e000770, https://doi.org/10.1136/esmoopen-2020-000770.
[26] D. Cabañero, E. Villalba-Riquelme, G. Fernández-Ballester, A. Fernández-Carvajal, A. Ferrer-Montiel, ThermoTRP channels in pain sexual dimorphism: new insights for drug intervention, Pharm. Ther. 240 (2022) 108297, https://doi.org/10.1016/j.pharmthera.2022.108297.
[27] K. Sałat, Chemotherapy-induced peripheral neuropathy: part 1-current state of knowledge and perspectives for pharmacotherapy, Pharmacol. Rep. 72 (3) (2020) 486–507, https://doi.org/10.1007/s43440-020-00109-y.
[28] D. Mizrahi, S.B. Park, T. Li, H.C. Timmins, T. Triinh, K. Au, E. Battaglini, D. Wyld, R.D. Henderson, P. Grimison, H. Ke, P. Geelan-Small, J. Marker, B. Wall, D. Goldstein, Hemoglobin, body mass index, and age as risk factors for Paclitaxel- and Oxaliplatin-Induced peripheral neuropathy, JAMA Netw. Open 4 (2) (2021) e2036695, https://doi.org/10.1001/jamanetworkopen.2020.36695.
[29] G. Balboni, I. Lazzari, C. Trapella, L. Negri, R. Lattanzi, E. Giannini, A. Nicotra, P. Melchiorri, S. Visentin, C.D. Nuccio, S. Salvadori, Triazine compounds as antagonists at Bv8-prokineticin receptors, J. Med. Chem. 51 (23) (2008) 7635–7639, https://doi.org/10.1021/jm800854e.
[30] E. Giannini, R. Lattanzi, A. Nicotra, A.F. Campese, P. Grazioli, I. Screpanti, G. Balboni, S. Salvadori, P. Sacerdote, L. Negri, The chemokine Bv8/prokineticin 2 is up-regulated in inflammatory granulocytes and modulates inflammatory pain, Proc. Natl. Acad. Sci. 106 (34) (2009) 14646–14651, https://doi.org/10.1073/pnas.0903720106.
[31] A.T. Brini, G. Amodeo, L.M. Ferreira, A. Milani, S. Niada, G. Moschetti, S. Franchi, E. Borsani, L.F. Rodella, A.E. Panerai, P. Sacerdote, Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain, Sci. Rep. 7 (1) (2017) 9904, https://doi.org/10.1038/s41598-017-09487-5.
[32] J.C.S. Mapplebeck, S. Beggs, M.W. Salter, Sex differences in pain: a tale of two immune cells, Pain 157 (1) (2016) S2–S6, https://doi.org/10.1097/j.pain.0000000000000389.
[33] M. Tsuda, Y. Shigemoto-Mogami, S. Koizumi, A. Mizokoshi, S. Kohsaka, M. W. Salter, K. Inoue, P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury, Nature 424 (6950) (2003) 778–783, https://doi.org/10.1038/nature01786.
[34] L. Ulmann, J.P. Hatcher, J.P. Hughes, S. Chaumont, P.J. Green, F. Conquet, G. N. Buell, A.J. Reeve, I.P. Chessell, F. Rassendren, Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain, J. Neurosci. 28 (44) (2008) 11263–11268, https://doi.org/10.1523/JNEUROSCI.2308-08.2008.
[35] R.E. Sorge, J.C. Mapplebeck, S. Rosen, S. Beggs, S. Taves, J.K. Alexander, L. J. Martin, J.S. Austin, S.G. Sotocinal, D. Chen, M. Yang, X.Q. Shi, H. Huang, N. J. Pillon, P.J. Bilan, Y. Tu, A. Klip, R.R. Ji, J. Zhang, M.W. Salter, J.S. Mogil, Different immune cells mediate mechanical pain hypersensitivity in Male and female mice, Nat. Neurosci. 18 (8) (2015) 1081–1083, https://doi.org/10.1038/nn.4053.
[36] S. Parsania, M. Shabani, K. Moazzami, M. Razavinasab, M.H. Larizadeh, M. Nazeri, M. Asadi-Shekaari, M. Kermani, Gender difference in motor impairments induced by chronic administration of vinblastine, Iran. J. Basic Med. Sci. 17 (6) (2014) 433–440.
[37] H. Naji-Esfahani, G. Vaseghi, L. Safaeian, A.A. Pilehvarian, A. Abed, M. Rafieian-Kopaei, Gender differences in a mouse model of chemotherapy-induced neuropathic pain, Lab. Anim. 50 (1) (2016) 15–20, https://doi.org/10.1177/0023677215575863.
[38] D.J. Goode, E.E. Whitaker, N.E. Mecum, Ovariectomy increases paclitaxel-induced mechanical hypersensitivity and reduces anti-inflammatory CD4+ t cells in the dorsal root ganglion of female mice, J. Neuroimmunol. 367 (2022) 577878, https://doi.org/10.1016/j.jneuroim.2022.577878.
[39] J. Zhang, J.M. Junigan, R. Trinh, A. Kavelaars, C.J. Heijnen, P.M. Grace, HDAC6 inhibition reverses Cisplatin-Induced mechanical hypersensitivity via tonic delta opioid receptor signaling, J. Neurosci. 42 (42) (2022) 7862–7874, https://doi.org/10.1523/JNEUROSCI.1182-22.2022.
[40] S.A. Woller, M. Corr, T.L. Yaksh, Differences in cisplatin-induced mechanical allodynia in Male and female mice, Eur. J. Pain. 19 (10) (2015) 1476–1485, https://doi.org/10.1002/ejp.679.
[41] R.E. Sorge, M.L. LaCroix-Fralish, A.H. Tuttle, S.G. Sotocinal, J.S. Austin, J. Ritchie, M.L. Chanda, A.C. Graham, L. Topham, S. Beggs, M.W. Salter, J.S. Mogil, Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in Male but not female mice, J. Neurosci. 31 (43) (2011) 15450–15454, https://doi.org/10.1523/JNEUROSCI.3859-11.2011.
[42] N. Iguchi, S.L. Hecht, D. Gao, D.T. Wilcox, A.P. Malykhina, N.G. Cost, Sexual dimorphic impacts of systemic vincristine on lower urinary tract function, Sci. Rep. 12 (1) (2022) 5113, https://doi.org/10.1038/s41598-022-08585-3.
[43] S. Lehnardt, L. Massillon, P. Follett, F.E. Jensen, R. Ratan, P.A. Rosenberg, J. J. Volpe, T. Vartanian, Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway, PNAS 100 (14) (2003) 8514–8519, https://doi.org/10.1073/pnas.1432609100.
[44] M.W. Alhadlaq, W. Masocha, Microglia and p38 MAPK inhibitors suppress development of mechanical allodynia in both sexes in a mouse model of Antiretroviral-Induced neuropathic pain, Int. J. Mol. Sci. 24 (16) (2023) 12805, https://doi.org/10.3390/ijms241612805.
[45] M. Campero, J. Serra, H. Bostock, J.L. Ochoa, Slowly conducting afferents activated by innocuous low temperature in human skin, J. Physiol. 535 (Pt 3) (2001) 855–865, https://doi.org/10.1111/j.1469-7793.2001.t01-1-00855.x.
[46] M. Campero, H. Bostock, Unmyelinated afferents in human skin and their responsiveness to low temperature, Neurosci. Lett. 470 (3) (2010) 188–192, https://doi.org/10.1016/j.neulet.2009.06.089.
[47] E.K. Lippoldt, S. Ongun, G.K. Kusaka, D.D. McKemy, Inflammatory and neuropathic cold allodynia are selectively mediated by the neurotrophic factor receptor GFRα3, PNAS 113 (16) (2016) 4506–4511, https://doi.org/10.1073/pnas.1603294113.
[48] K. Kobayashi, T. Fukuoka, K. Obata, H. Yamanaka, Y. Dai, A. Tokunaga, K. Noguchi, Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors, J. Comp. Neurol. 493 (4) (2005) 596–606, https://doi.org/10.1002/cne.20794.
[49] T.S. Jensen, N.B. Finnerup, Allodynia and hyperalgesia in neuropathic pain: clinical manifestations and mechanisms, Lancet Neurol. 13 (9) (2014) 924–935, https://doi.org/10.1016/S1474-4422(14)70102-4.
[50] M. Nazıroğlu, N. Braidy, Thermo-Sensitive TRP channels: novel targets for treating Chemotherapy-Induced peripheral pain, Front. Physiol. 8 (2017) 1040, https://doi.org/10.3389/fphys.2017.01040.
[51] R. Srinivasan, D. Wolfe, J. Goss, S. Watkins, W.C. de Groat, A. Sculptoreanu, J. C. Glorioso, Protein kinase c epsilon contributes to basal and sensitizing responses of TRPV1 to capsaicin in rat dorsal root ganglion neurons, Eur. J. Neurosci. 28 (7) (2008) 1241–1254, https://doi.org/10.1111/j.1460-9568.2008.06438.x.
[52] C. Goswami, J. Kuhn, O.A. Dina, G. Fernández-Ballester, J.D. Levine, A. Ferrer-Montiel, T. Hucho, Estrogen destabilizes microtubules through an ion-conductivity-independent TRPV1 pathway, J. Neurochem. 117 (6) (2011) 995–1008, https://doi.org/10.1111/j.1471-4159.2011.07270.x.
[53] E. Falvo, S. Diviccaro, R.C. Melcangi, S. Giatti, Physiopathological role of neuroactive steroids in the peripheral nervous system, Int. J. Mol. Sci. 21 (23) (2020) 9000, https://doi.org/10.3390/ijms21239000.
[54] I. Roglio, R. Bianchi, F. Camozzi, V. Carozzi, I. Cervellini, D. Crippa, G. Lauria, G. Cavaletti, R.C. Melcangi, Docetaxel-induced peripheral neuropathy: protective effects of dihydroprogesterone and progesterone in an experimental model, J. Peripher. Nerv. Syst. 14 (1) (2009) 36–44, https://doi.org/10.1111/j.1529-8027.2009.00204.x.
[55] L. Meyer, C. Patte-Mensah, O. Taleb, A.G. Mensah-Nyagan, Cellular and functional evidence for a protective action of neurosteroids against vincristine chemotherapy-induced painful neuropathy, Cell. Mol. Life Sci. CMLS 67 (17) (2010) 3017–3034, https://doi.org/10.1007/s00018-010-0372-0.
[56] C. Jung, C. Fandos, I.M. Lorenzo, C. Plata, J. Fernandes, G.G. Gené, E. Vázquez, M. A. Valverde, The progesterone receptor regulates the expression of TRPV4 channel, Pflug. Arch. 459 (1) (2009) 105–113, https://doi.org/10.1007/s00424-009-0706-7.
[57] M. Ortíz-Rentería, R. Juárez-Contreras, R. González-Ramírez, L.D. Islas, F. Sierra-Ramírez, I. Llorente, S.A. Simon, M. Hiriart, T. Rosenbaum, S.L. Morales-Lázaro, TRPV1 channels and the progesterone receptor Sig-1R interact to regulate pain, Proc. Natl. Acad. Sci. 115 (7) (2018) E1657–E1666, https://doi.org/10.1073/pnas.1715972115.
[58] C. Martucci, S. Franchi, E. Giannini, H. Tian, P. Melchiorri, L. Negri, P. Sacerdote, Bv8, the amphibian homologue of the mammalian prokineticins, induces a proinflammatory phenotype of mouse macrophages, Br. J. Pharm. 147 (2) (2006) 225–234, https://doi.org/10.1038/sj.bjp.0706467.
[59] S. Franchi, E. Giannini, D. Lattuada, R. Lattanzi, H. Tian, P. Melchiorri, L. Negri, A. E. Panerai, P. Sacerdote, The prokineticin receptor agonist Bv8 decreases IL-10 and IL-4 production in mice splenocytes by activating prokineticin receptor-1, BMC Immunol. 9 (2008) 60, https://doi.org/10.1186/1471-2172-9-60.