

Matrix Metalloproteinase Inhibitor COL-3 Prevents the Development of Paclitaxel-Induced Hyperalgesia in Mice

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Key Words

Paclitaxel · Chemotherapy-induced peripheral neuropathy · Pain · Prevention · Chemically modified tetracycline · COL-3 · Matrix metalloproteinase inhibitor · Cytokine · Chemokine

Abstract

Objective: To study the potential of chemically modified tetracycline-3 (COL-3), a potent matrix metalloproteinase (MMP) inhibitor, to protect against the development of paclitaxel-induced painful neuropathy and its immunomodulatory effects. **Materials and Methods:** The reaction latency to thermal stimuli (hot plate test) of female BALB/c mice was recorded before and after treatment with paclitaxel (2 mg/kg i.p.), paclitaxel plus COL-3 (4, 20 or 40 mg/kg p.o.) or their vehicles for 5 consecutive days. Gene transcripts of CD11b (marker for microglia), 5 cytokines (IFN- γ , IL-1 β , IL-6, IL-10 and TNF- α) and 3 chemokines (CCL2, CXCL10 and CX3CL1) were quantified by real-time PCR in the brains, spinal cords and spleens of mice sacrificed on day 7 after treatment. **Results:** Treatment with paclitaxel reduced the reaction latency time to thermal stimuli (thermal hyperalgesia) for 4 weeks, with maximum effect on days 7 and 10. The coadministration of paclitaxel with COL-3 40 mg/kg, but not lower doses, prevented the development of paclitaxel-induced thermal hyperalgesia. Treatment with paclitaxel alone or coadministration with COL-3 increased CD11b transcript levels in the

brain but not in the spinal cord. Treatment with paclitaxel reduced IL-6 transcript levels in the spinal cord but did not alter the transcript levels of other cytokines or chemokines in the brain, spinal cord or spleen. The coadministration of COL-3 with paclitaxel significantly increased the transcript levels of IL-6 in the spleen and decreased CX3CL1 transcripts in the brain in comparison to treatment with paclitaxel alone.

Conclusion: Our results indicate that the MMP inhibitor COL-3 protected against paclitaxel-induced thermal hyperalgesia and, thus, could be useful in the prevention of chemotherapy-induced painful neuropathy.

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Introduction

Taxanes such as paclitaxel (Taxol[®]), which are mitotic spindle inhibitors [1], are fundamental in the treatment of breast cancer and other solid tumors. However, their use is hampered by the development of dose-limiting painful peripheral neuropathy [2, 3]. Paclitaxel preferentially impairs sensory fibers and produces neuropathy in the distal extremities, hands and feet, presenting in a glove-stocking pattern [4]. This peripheral neuropathy presents as a chronic painful neuropathic syndrome in some patients.

In rodent models paclitaxel-induced neuropathic pain (PINP) manifests as mechanical allodynia or thermal

hyperalgesia [3, 5–7]. Various pathogenetic mechanisms have been proposed to play a role in the development of PINP, including glial cell activation in the central nervous system [8, 9]. Cytokines might also be involved in the pathogenesis of PINP since paclitaxel has been shown to induce cytokine expression [10, 11] and the administration of cytokines such as IL-6 and IL-10 has been reported to prevent the development of or attenuate PINP [5, 11]. Recent studies implicate matrix metalloproteinases (MMPs) and proteinase-activated receptors in the development of PINP [12, 13].

Taking into consideration the recently suggested role of MMPs in the development of PINP, we evaluated the potential of COL-3 (6-demethyl-6-deoxy-4-dedimethylaminotetracycline), also known as CMT-3, a potent MMP inhibitor [14–16], to prevent the development of PINP.

Animals and Methods

Animals

All animals (n = 166) used in this study were female BALB/c mice (8–12 weeks old; 20–30 g) supplied by the Animal Resources Center at the Health Sciences Center, Kuwait University, Kuwait. The mice were kept in temperature-controlled ($24 \pm 1^\circ\text{C}$) rooms with food and water ad libitum. All experiments were performed during the same period of the day (8:00 a.m. to 4:00 p.m.) to exclude diurnal variations in pharmacological effects. The animals were handled in compliance with European Communities Council Directive 86/609 for the care of laboratory animals and ethical guidelines for research in experimental pain with conscious animals [17].

Drug Treatment and Assessment of Thermal Nociception

Paclitaxel (Tocris, Bristol, UK) was dissolved in a solution made up of 50% Cremophor EL and 50% absolute ethanol to a concentration of 6 mg/ml and stored at -20°C for a maximum of 14 days. It was then diluted in normal saline (NaCl 0.9%) to a final concentration of 0.2 mg/ml just before administration. The vehicle for paclitaxel was diluted at the time of injection with normal saline in the same proportion as the paclitaxel solution. Paclitaxel 2 mg/kg or its vehicle were administered to the mice intraperitoneally, in a volume of 10 ml/kg, once per day for 5 consecutive days. This treatment regimen has been reported to produce painful neuropathy in mice [6].

COL-3 (a gift from Galderma, Research and Development SNC, Les Templier, France) was dissolved in 1% methylcellulose and administered to mice by oral gavage in a volume of 12.5 ml/kg body mass. COL-3 (4–40 mg/kg) was coadministered with paclitaxel or its vehicle daily for 5 days. The mice were assessed for the development of neuropathic pain (thermal hyperalgesia) and those that received paclitaxel plus COL-3 were compared with the mice treated with the paclitaxel plus vehicle (for COL-3) only.

Reaction latencies to the hot plate test were measured before treatment (baseline latency) and on days 7, 10, 14, 17, 21 and 28 after the first injection of drugs (paclitaxel or COL-3). Briefly, the

mice were individually placed on a hot plate (Panlab SL, Barcelona, Spain) with the temperature adjusted to $55 \pm 1^\circ\text{C}$. The time to the first sign of nociception, paw licking, flinching or jump response to avoid the heat was recorded and the animal immediately removed from the hot plate. A cutoff period of 20 s was maintained to avoid damage to the paws. The observer (S.S.P.) was blinded to the treatment the animal received. The percentage change in reaction latency was calculated as follows: [(response latency after drug treatment – pretreatment baseline latency)/pretreatment baseline latency] $\times 100$.

Gene Expression Analysis by Real-Time PCR

Gene transcripts of CD11b (a marker for microglia), 5 cytokines (IFN- γ , IL-1 β , IL-6, IL-10 and TNF- α) and 3 chemokines (CCL2, CXCL10 and CX3CL1) were quantified by real-time PCR in the brains, spinal cords and spleens dissected out from the mice sacrificed at 7 days after the first administration of the vehicle, paclitaxel or paclitaxel plus COL-3 40 mg/kg. Total RNA was extracted from half of the fresh frozen brains and reverse-transcribed as described previously [18]. The transcript levels were quantified on an ABI Prism[®] 7500 sequence detection system (Applied Biosystems, Foster City, Calif., USA) as previously described [19]. The primer sequences which were used, listed in table 1, were ordered from Invitrogen (Life Technologies, Carlsbad, Calif., USA). Threshold cycle (C_t) values for all cDNA samples were obtained and the amount of transcripts of individual animal samples (n = 4–6 per group) was normalized to cyclophilin (ΔC_t). The relative amount of target gene transcripts was calculated using the $2^{-\Delta\Delta C_t}$ method as described previously [20]. These values were then used to calculate the mean and standard error (SEM) of the relative expression of the target gene mRNA in the brain of the drug- and vehicle-treated mice.

Statistical Analyses

Statistical analyses were performed using the unpaired t test with Welch's correction, one-way ANOVA followed by Newman-Keuls multiple comparison test or two-way repeated measures ANOVA followed by Bonferroni posttests. The differences were considered significant at $p < 0.05$. The results in the text and figures are expressed as the means \pm SEM.

Results

Paclitaxel-Induced Thermal Hyperalgesia

Paclitaxel produced a significant reduction in response latency time to thermal stimuli (thermal hyperalgesia) from 7 to 28 days after the first drug administration compared to the baseline latency and from 7 to 21 days compared to the vehicle-only-treated animals in the hot plate test ($p < 0.01$; fig. 1).

COL-3 Prevents the Development of Paclitaxel-Induced Thermal Hyperalgesia

The treatment of the naïve mice with COL-3 40 mg/kg by oral gavage daily for 5 consecutive days did not

Table 1. PCR primer sequences of cyclophilin, CD11b, cytokines and chemokines

Gene	Polarity	Sequence 5' to 3'	GenBank No.
Cyclophilin	Sense	GCTTTTCGCCGCTTGCT	X52803
	Antisense	CTCGTCATCGGCCGTGAT	
CD11b	Sense	TGCTTACCTGGGTTATGCTTCTG	NM_008401
	Antisense	CCGAGGTGCTCCTAAAACCA	
IFN- γ	Sense	ACAATGAACGCTACACACTGCAT	NM_008337
	Antisense	TGGCAGTAACAGCCAGAAACA	
IL-1 β	Sense	TGGTGTGTGACGTTCCCAT	NM_010554
	Antisense	CAGCACGAGGCTTTTTTGTG	
IL-6	Sense	ACAAGTCGGAGGCTTAATTACACAT	NM_031168
	Antisense	TTGCCATGCACAACCTCTTTTC	
IL-10	Sense	CAGCCGGGAAGACAATAACTG	NM_010548
	Antisense	CCGACAGCTCTAGGAGCATGT	
TNF- α	Sense	GGCTGCCCCGACTACGT	NM_013693
	Antisense	GACTTTCCTGGTATGAGATAGCAA	
CCL2	Sense	GGCTCAGCCAGATGCAGTTAAC	NM_011333
	Antisense	CCTACTCATTGGGATCATCTTGCT	
CXCL10	Sense	GACGGTCCGCTGCAACTG	NM_021274
	Antisense	GCTTCCCTATGGCCCTCATT	
CXC3CL1	Sense	ATTGTCTGGAGACGACACAGC	NT_078575
	Antisense	TTGCCACCATTTTTAGTGAGGG	

GenBank accession numbers of sequences used for primer design.

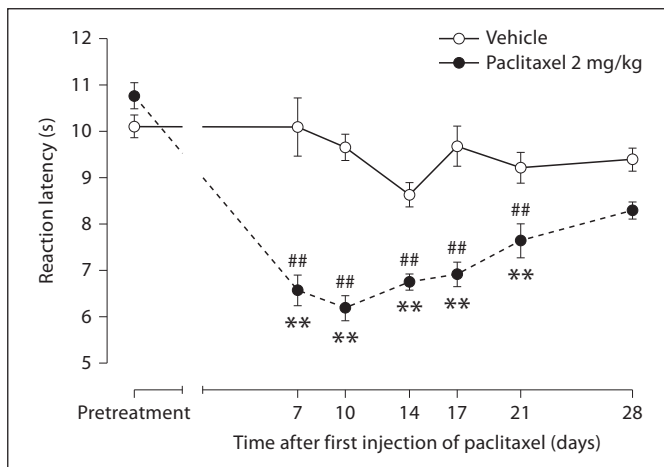


Fig. 1. Paclitaxel-induced thermal hyperalgesia in BALB/c mice. Time course of the reaction latency time to the hot plate test after the administration of paclitaxel 2 mg/kg or its vehicle. Each point represents the mean \pm SEM of the values obtained from 14–16 animals. ** $p < 0.01$ compared to drug vehicle on the same day after treatment; # $p < 0.01$ compared to pretreatment values.

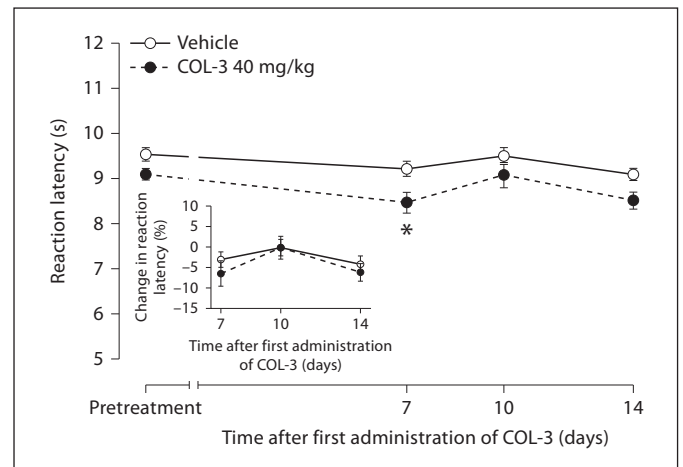
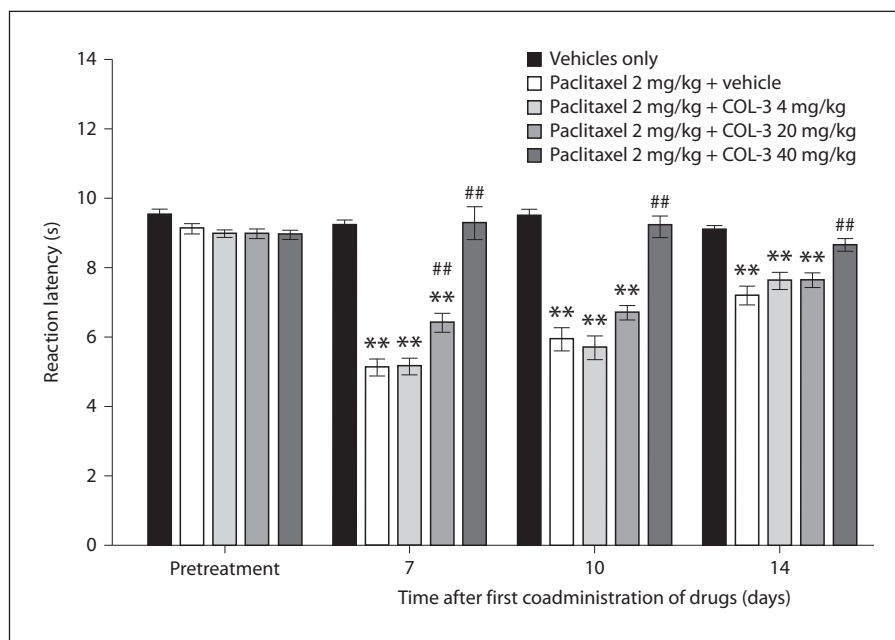


Fig. 2. Time course of the reaction latency time to the hot plate test after the administration of COL-3 40 mg/kg or its vehicle. Each point represents the mean \pm SEM of the values obtained from 19–21 animals. There were no statistically significant differences in the percentage change in reaction latency between the COL-3-treated and the vehicle-treated animals. * $p < 0.05$ compared to drug vehicle on the same day after treatment.

Fig. 3. Coadministration of COL-3 with paclitaxel protects against the development of paclitaxel-induced thermal hyperalgesia. Effects of coadministration of paclitaxel with COL-3 on the development of paclitaxel-induced thermal hyperalgesia in BALB/c mice. Each point represents the mean \pm SEM of the values obtained from 17–23 animals. ** $p < 0.01$ compared to control mice; ## $p < 0.01$ compared to mice treated with paclitaxel plus vehicle.



cause any significant changes to the reaction latency compared to the pretreatment baseline values ($p > 0.05$; fig. 2). The COL-3-treated mice showed a slightly lower reaction latency compared to the vehicle-treated mice only on day 7 ($p < 0.05$; fig. 2). However, the percentage changes from pretreatment latencies were not different between the vehicle-treated and the COL-3-treated mice ($p > 0.05$; fig. 2 insert), indicating that COL-3 did not affect the mice's reaction latencies to thermal nociception.

The mice treated with paclitaxel plus COL-3 (40 mg/kg) had reaction latency times similar to the vehicle-only-treated control animals, which were significantly higher than those of the mice treated with paclitaxel only ($p < 0.01$; fig. 3). However, the reaction latency times of the mice treated with paclitaxel plus lower doses of COL-3 (4 or 20 mg/kg) were significantly lower than those of the vehicle-only-treated control animals ($p < 0.01$), but the reaction latency of the mice treated with paclitaxel plus COL-3 at 20 mg/kg was significantly higher on day 7 after the first drug administration than those treated with paclitaxel alone ($p < 0.01$; fig. 3).

Effects of COL-3 on Cytokine, Chemokine and CD11b Transcript Levels during Paclitaxel-Induced Thermal Hyperalgesia

Treatment with either paclitaxel alone or the coadministration of paclitaxel plus COL-3 caused a significant

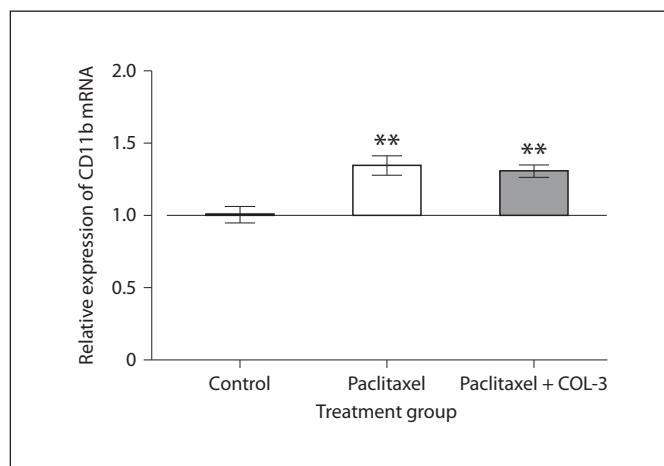


Fig. 4. Effects of coadministration of paclitaxel with COL-3 on relative CD11b mRNA expression in the brains of BALB/c mice on day 7 after the first administration of drugs. Each point represents the mean \pm SEM of the values obtained from 5–6 animals. ** $p < 0.01$ compared to control mice.

increase in CD11b transcript levels (a marker for microglia activation) in the brain compared to the vehicle-only-treated controls ($p < 0.01$; fig. 4). However, treatment with paclitaxel or the coadministration of paclitaxel plus COL-3 did not significantly affect the levels of CD11b transcripts in the spinal cord (data not shown).

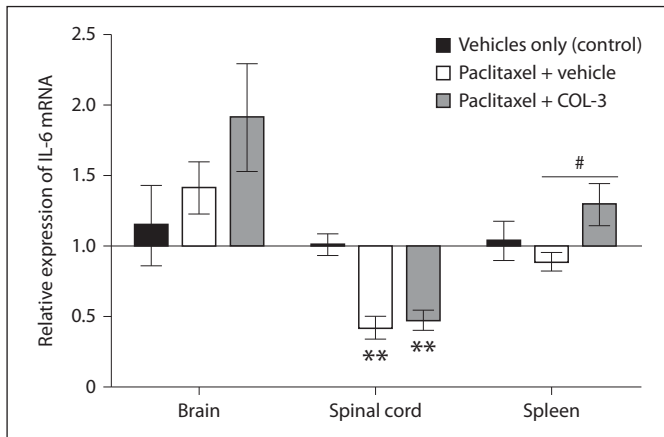


Fig. 5. Effects of coadministration of paclitaxel with COL-3 on relative IL-6 mRNA expression in the brains, spinal cords and spleens of BALB/c mice on day 7 after the first administration of drugs. Each point represents the mean \pm SEM of the values obtained from 4–6 animals. ** $p < 0.01$ compared to control mice; # $p < 0.05$ compared to mice treated with paclitaxel plus vehicle.

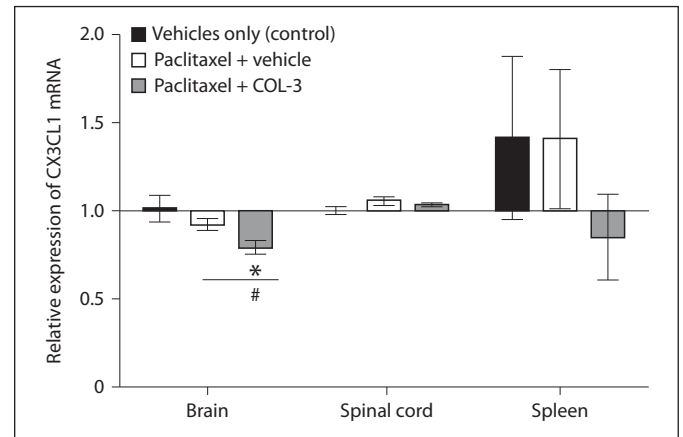


Fig. 6. Effects of coadministration of paclitaxel with COL-3 on relative CX3CL1 mRNA expression in the brains, spinal cords and spleens of BALB/c mice on day 7 after the first administration of drugs. Each point represents the mean \pm SEM of the values obtained from 4–6 animals. * $p < 0.05$ compared to control mice; # $p < 0.05$ compared to mice treated with paclitaxel plus vehicle.

Treatment with either paclitaxel alone or the coadministration of paclitaxel plus COL-3 caused a significant reduction in IL-6 transcript levels in the spinal cord compared to the vehicle-only-treated controls ($p < 0.01$; fig. 5). The spleens of the mice treated with paclitaxel plus COL-3 had significantly higher levels of IL-6 transcripts compared to those of the mice treated with paclitaxel only ($p < 0.05$; fig. 5). However, treatment with paclitaxel or the coadministration of paclitaxel plus COL-3 did not significantly affect the levels of the transcripts of the other cytokines (IFN- γ , IL-1 β , IL-10 and TNF- α) in the brain, spinal cord or spleen (data not shown).

The brains of the mice treated with paclitaxel plus COL-3 had significantly low levels of CX3CL1 transcripts compared to those of the mice treated with paclitaxel plus vehicle or vehicle only ($p < 0.05$; fig. 6). On the other hand, treatment of the mice with paclitaxel alone or the coadministration of paclitaxel plus COL-3 did not significantly affect the levels of the transcripts of the other chemokines (CCL2 and CXCL10) in the brain, spinal cord or spleen (data not shown).

Discussion

This study showed for the first time that the MMP inhibitor COL-3 protects against the development of paclitaxel-induced thermal hyperalgesia. It also provided evi-

dence that the coadministration of COL-3 with paclitaxel increased IL-6 transcripts in the spleen and decreased the transcripts of CX3CL1 in the brains of mice compared to treatment with paclitaxel only.

Paclitaxel is a standard treatment, often in combination with carboplatin, for various cancers including lung, breast and ovarian cancer. However, its use is limited by the development of peripheral neuropathy which is painful in some patients [4]. Treatment with paclitaxel has been previously shown to produce painful neuropathy, including thermal hyperalgesia, in mice [6].

Currently, there are no clinically available effective treatments for the prevention or treatment of this dose-limiting painful neuropathy. In order to find possible drugs for the prevention of PINP considerable research is being undertaken to understand its pathophysiology. The mechanism for the development of PINP is not clear, but various factors have been proposed, including the disruption of microtubule functions in axons, the generation of free radicals, glial cell activation, cytokine production and the upregulation of MMPs and activation of proteinase-activated receptors [3, 8–13, 21].

We found that COL-3, a potent MMP inhibitor, prevented the development of paclitaxel-induced thermal hyperalgesia, suggesting that MMPs are a plausible target for the prevention of PINP. Minocycline, a tetracycline antibiotic with anti-inflammatory activities including the inhibition of MMPs, though less potent than

COL-3, has been reported to prevent the development of PINP, most probably via its inhibitory effects on glial cell activation and macrophage infiltration into the dorsal root ganglion [9, 21]. However, the administration of minocycline over long periods of time may result in the emergence of bacterial resistance to tetracycline antibiotics and also lead to undesirable side effects through the killing of commensal bacteria. COL-3, on the other hand, does not have antibiotic activities because it lacks the dimethylamino group from the carbon-4 position, the side chain, which is required for the antimicrobial activity of tetracyclines [14, 22]. Apart from COL-3 being an MMP inhibitor, recent data suggest that it can also inhibit serine proteinases [23]. This activity of the drug could also contribute to its protective effects against the development of PINP, since serine proteinase-activated receptors have recently been implicated in the development of PINP [12].

Glial cells, cytokines and chemokines have also been implicated in the development of neuropathic pain including PINP [10, 11]. In BALB/c mice, we observed an increase in CD11b transcripts in the brains, but not in the spinal cords, of paclitaxel-treated animals, which was not affected by the coadministration with COL-3. These data suggest that the protective effects of COL-3 against the development of PINP were not mediated via modulating microglia activity in the brain or spinal cord. We did not observe an increase in cytokines in the spleen, brain or spinal cord after treatment with paclitaxel. This is in agreement with the study by Zhang et al. [9], where no increase was observed in the proinflammatory cytokine levels in the spinal cords of animals with PINP. Interestingly, the coadministration of COL-3 with paclitaxel increased IL-6 transcripts in the spleen. However, the reduced expression of IL-6 transcripts in the spinal cords of the mice treated with paclitaxel alone was not prevented by the coadministration with COL-3. This suggests that the protective effects of COL-3 against the development of PINP could have been partly due to an increase of IL-6 in the periphery, but not in the spinal cord. The administration of IL-6 has been reported to protect against the development of PINP [5]. However, IL-6 is a cytokine that has pleiotropic activity, including neurotrophic and pro- and anti-inflammatory activities depending on the conditions [24]. Another interesting observation was the specific decrease in the transcripts of the chemokine CX3CL1 (also known as fractalkine) in the brains of the mice treated with both COL-3 and paclitaxel. CX3CL1 has been reported to mediate neuropathic pain [25]. Thus, the reduction of CX3CL1 expres-

sion could also have contributed to the protective effects of COL-3 against the development of PINP.

COL-3 also has antitumor activities [26, 27] and it has been evaluated in phase II clinical trials for the treatment of cancers such as sarcomas [28–30]. Interestingly, the dose of COL-3, 40 mg/kg, which we found to be effective for preventing paclitaxel-induced thermal hyperalgesia, was closer to the dose of COL-3 used for treating sarcomas, 50 mg/kg [28, 29]. Taking into consideration that COL-3 shows potential to improve the tolerability of paclitaxel, it would also be worth evaluating the possibility of increased efficacy against tumors by combining COL-3 with paclitaxel. If effective, this would present a double advantage of reducing side effects and increasing antitumor efficacy.

Conclusion

Our results showed that the MMP inhibitor COL-3 prevented the development of paclitaxel-induced thermal hyperalgesia. Therefore, this drug warrants further research as a potential candidate to be used in combination with paclitaxel to prevent the development of painful peripheral neuropathy.

Acknowledgements

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