Chemotherapy-induced peripheral neuropathy (CIPN) arises due to neurotoxicity produced by chemotherapy drugs such as paclitaxel and oxaliplatin. The lack of a blood-nerve barrier in the dorsal root ganglion (DRG) allows for accumulation of these chemotherapy drugs resulting in neuronal toxicity. This often results in debilitating sensory and motor deficits that are not effectively prevented or alleviated by existing therapeutic interventions. Recent studies have demonstrated the therapeutic effects of Meteorin, a neurotrophic factor, in reversing neuropathic pain in rodent models of peripheral nerve injury induced by physical trauma. Here, we sought to investigate the potential antinociceptive effects of recombinant mouse Meteorin (rmMeteorin) using a paclitaxel-induced peripheral neuropathy model in male and female mice. Paclitaxel treatment (4 × 4 mg/kg, i.p.) induced hind paw mechanical hypersensitivity by day 8 after treatment. Thereafter, in a reversal dosing paradigm, five repeated injections of rmMeteorin (0.5 and 1.8 mg/kg s.c. respectively) administered over 9 days produced a significant and long-lasting attenuation of mechanical hypersensitivity in both sexes. Additionally, administration of rmMeteorin (0.5 and 1.8 mg/kg), initiated before and during paclitaxel treatment (prevention dosing paradigm), reduced the establishment of hind paw mechanical hypersensitivity. Repeated systemic administration of rmMeteorin in both dosing paradigms decreased histochemical signs of satellite glial cell reactivity as measured by glutamine synthetase and connexin 43 protein expression in the dorsal root ganglion. Additionally, in the prevention administration paradigm rmMeteorin had a protective effect against paclitaxel-induced loss of intraepidermal nerve fibers. Our findings indicate that rmMeteorin has a robust and sustained antinociceptive effect in the paclitaxel-induced peripheral neuropathy model and the development of recombinant human Meteorin could be a novel and effective therapeutic for chemotherapy-induced peripheral neuropathy treatment.

**Perspective:** Chemotherapy neuropathy is a major clinical problem that decreases quality of life for cancer patients and survivors. Our experiments demonstrate that Meteorin treatment alleviates pain-related behaviors, and signs of neurotoxicity in a mouse model of paclitaxel neuropathy.

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Peripheral Neuropathic Pain

hyperexcitability and neuropathic pain.\textsuperscript{11,30,31} Characteristics of CIPN include numbness, tingling, hypersensitivity to cold and mechanical stimuli, and burning sensations in the hands and feet.\textsuperscript{5,20} It affects around 30-40% of cancer patients and can continue to persist even after cessation of treatment.\textsuperscript{41} Currently, there are no FDA-approved drugs for the treatment of CIPN. Paclitaxel which can induce neuropathy as a dose-limiting side effect accumulates in the DRG and causes direct neurotoxic effects such as axonal impairment and neuronal mitochondrial damage.\textsuperscript{4,29} Approximately 25% of patients with paclitaxel-induced neuropathy discontinue their treatment or reduce their dosage primarily due to pain.\textsuperscript{40} Hence, identifying a novel drug for the treatment of paclitaxel neuropathy would be hugely beneficial and be expected to improve both survival probability and the quality of life for cancer patients.

Neurotrophic factors promote growth, survival, differentiation, and maintenance of cells of the nervous system. These factors can promote a therapeutic effect in many neurodegenerative disorders and also in neuropathic pain.\textsuperscript{34,38} Gliarial-derived neurotrophic factor (GDNF), neurturin, and artemin belong to a transforming growth factor-\beta superfamily subgroup. GDNF was the first gliial cell-derived neurotrophic factor to show neuroprotection, and it signals through a GPI linked to the RET tyrosine kinase.\textsuperscript{45} Artemin has been shown to support the survival of sensory neurons in culture through interacting with a GFR-a3 receptor primarily expressed in the peripheral nervous system.\textsuperscript{10} GDNF administration also reverses mechanical hypersensitivity in rats with spinal nerve ligation.\textsuperscript{2,3} These and other studies on growth factors for pain treatment suggest great potential for antinociceptive effects, and even disease modification, but no treatments in this class have so far been approved for pain relief.\textsuperscript{25,36} Treatments sequestering growth factors have been tested in clinical trials for pain. Anti-NGF therapeutics achieved robust superiority over placebo in many clinical trials,\textsuperscript{6,7,18,19,21} but these therapeutics have not been approved for human use due to side-effects.

Meteorin is a neurotrophic factor which produces long-lasting antinociceptive effects in rodent models of peripheral nerve injury induced by physical trauma.\textsuperscript{16,47} It is expressed in the central and peripheral nervous system, with high expression levels in the DRG in humans and mice, where it is likely expressed by DRG neurons and satellite glial cells based on single cell sequencing experiments.\textsuperscript{37,43,48} Meteorin promotes neurite outgrowth in neurons, including DRG neurons where it has an effect on small and intermediate-sized neurons, which are presumably nociceptors.\textsuperscript{17,32} Meteorin also promotes glial cell differentiation via the activation of the JAK-STAT pathway, but the underlying receptor-mediated mechanisms leading to these effects are still unclear.\textsuperscript{22} Two previous studies have demonstrated the therapeutic effects of rmMeteorin in reversing neuropathic pain induced in the sciatic nerve injury in rats.\textsuperscript{16,47}

The goal of this study was to test the hypothesis that recombinant mouse Meteorin (rmMeteorin) would be able to prevent and reverse paclitaxel-induced peripheral neuropathic pain in male and female mice. In support of our hypothesis, we demonstrate that systemic administration of rmMeteorin (.5 mg/kg and 1.8 mg/kg) is efficacious in prevention and reversal paradigms in the mouse paclitaxel-induced neuropathy model with mechanical hypersensitivity as the primary endpoint. Administration of rmMeteorin also reduced the expression of satellite cell gliosis. Finally, rmMeteorin partially halted intraepidermal nerve fiber (IENF) loss in the prevention dosing paradigm. Overall, our findings demonstrate that the development of recombinant human Meteorin could be a novel and effective therapeutic for CIPN treatment.

Methods

Animals

ICR (CD-1) male and female mice were purchased from Envigo and maintained at the animal facility at the University of Texas at Dallas. Mice were group-housed (4 maximum) in cages with bedding material provided for enrichment with food and water available ad libitum in a 12:12 hours light-dark cycle. Room temperature was maintained at 21 to 22°C. Behavioral experiments were performed on mice ranging between 8 and 12 weeks old at the start of the experiment. All procedures were approved by the Institution Animal Care and Use Committee at the University of Texas at Dallas. Both male and female mice were used for behavioral testing in the reversal paradigm and only female mice were used for behavioral testing in the prevention paradigm. We made efforts described in the results to reduce the number of animals used in this study.

Injections

Administered drug was prepared by dissolving Paclitaxel in 50% EL Kolliphor (Sigma-Aldrich) and 50% ethanol and further diluted in sterile Dulbecco’s phosphates buffered saline (DPBS; Thermo Scientific). Mice were administrated 4 mg/kg every-other-day for 4 days for a cumulative dosage of 16 mg/kg or Vehicle control of 50% EL Kolliphor and 50% ethanol diluted in DPBS. Paclitaxel was injected intraperitoneally (i.p.) on days 2, 4, 6, and 8 using a 27-gauge needle.\textsuperscript{24} Starting on day 10, in a reversal paradigm, five subcutaneous (s.c.) injections of recombinant mouse Meteorin (rmMeteorin, R & D Systems #3475) either .5 mg/kg or 1.8 mg/kg or Vehicle (DPBS) was administered every other day for ten days using an Insulin syringe\textsuperscript{24} with a 30-gauge needle. For the prevention paradigm, s.c. rmMeteorin .5 or 1.8 mg/kg or Vehicle was administered before and then given intermittently between paclitaxel treatments on days 1, 3, 5, 7, and 9. The investigator was blinded to treatment during all the injections.

Mechanical Withdrawal Thresholds

Behavioral testing was performed after habituating mice for 2 hours to clear acryl behavior hambers before
beginning mechanical testing. Mechanical hypersensitivity was tested at baseline and every other day, with no injections or as indicated in the figures. Mechanical paw withdrawal threshold was tested with the up-down method using calibrated von Frey filaments (Stoelting) applied to the mid plantar surface of the left hind paw. A positive response was comprised of licking or immediate flicking of the hind paw upon application of the filament. The investigator was blinded on all days of testing.

On day 24, three mice per group were euthanized to harvest tissue for immunohistochemistry. Mechanical paw withdrawal thresholds continued to be assessed in the remaining mice until they had resolved back to a baseline level. Two mice were removed from the male cohort on day nine of the reversal paradigm as they were not hypersensitive after the last paclitaxel injection. Male behavior testing in the reversal paradigm was halted after day 21 and resumed on day 51 due to a mandatory COVID lockdown in March-April 2020. None of the mice were removed from the female cohorts of both paradigms.

Immunohistochemistry (IHC)

On day 24, three animals per group were anesthetized using 4% isoflurane and euthanized by decapitation. DRG and skin tissues were flash-frozen in Optimum cutting temperature (OCT) medium (Fisher Scientific) on dry ice. Twenty μm sections were cut on a cryostat and mounted onto SuperFrost Plus slides (Thermo Fisher Scientific). The sections were fixed in ice-cold 10% formalin for 15 minutes followed by incubation in an increasing percentage of ethanol 50%, 70%, 100%, for 5 minutes each. The fixed slides were transferred into blocking solution (10% Normal Goat Serum, .3% Triton-X 100 in .1 M PB) for 1 hour at room temperature. Sections were incubated in primary antibody (Table 1) diluted in blocking solution for 3 hours at room temperature or 4 °C overnight. They were then washed in .1 M PB followed by incubation in secondary antibody diluted in blocking solution for 1 hour at room temperature. The slides were washed again with .1 M PB followed by incubation with DAPI diluted in blocking solution for 5 minutes at room temperature. Lastly, the sections were washed in .1 M PB and cover-slipped using Prolong Gold Antifade (Thermo Fisher Scientific). Images were taken using an Olympus FluoView 3,000 confocal microscope and analyzed using CellSens (Olympus) software. All IHC images are a representative of a sample size of 3 animals per group.

### Table 1. List of antibodies used for immunohistochemistry

| Antibody                                      | Company                      | Catalog #      | Dilution |
|-----------------------------------------------|------------------------------|----------------|----------|
| Glutamine Synthetase polyclonal antibody      | Thermo fisher               | Cat # 11307-2-AP | 1:1,000  |
| Peripherin                                    | EnCor Biotechnology Inc      | Cat # CPCA-Peri | 1:1,000  |
| DAPI                                          | Cayman                       | Item no, 14285  | 1:5,000  |
| Connexin 43                                   | Cell Signaling Technologies  | Cat # 3512S    | 1:1,000  |
| PGP 9.5                                       | Cedarlane Labs               | CL7756AP-50    | 1:500    |
| IgG (H+L) Cross-Adsorbed Goat anti-Rabbit, Alexa Fluor® 555 | Fisher Scientific       | A21428         | 1:2,000  |
| IgY (H+L) Goat anti-Chicken, Alexa Fluor® 647  | Fisher Scientific           | A21449         | 1:2,000  |

Image analyses of the DRG sections were performed by drawing a region of interest (ROI) around each individual neuron and the mean grey intensity value was calculated in the targeted channel of interest. Average mean grey intensity was subtracted from the background fluorescence intensity calculated using the negative control (secondary antibody only without primary antibody) and normalized over the area surrounding each individual neuronal cell body.

Image analysis of IENF density on skin sections was done over the plantar surface of the hind paw by calculating the number of fibers crossing the basement membrane over the length of the epidermal membrane per millimeter. Three sections per animal were analyzed with 3 animals per group to calculate the IENF density.

Data and Statistical Analysis

Data analysis was done using Graphpad Prism 8.4.1. Statistical differences between groups were assessed using one-way ANOVA followed by Tukey multiple comparisons. P-values are reported in the figures and figure legends. Behavioral analysis was determined using mixed-effects 2-way ANOVA analysis followed by Tukey post hoc tests. The mixed-effect model was used to account for the missing values starting on day 24 when 4 animals per group were euthanized for IHC. P-values for main effects are given in the figure legends and full post hoc P-value tables are given in Supplementary Table 1. Effect sizes were determined by subtracting behavior scores from baseline values. Absolute values were summed from the beginning of rmMeteorin administration (from day 10 for the reversal paradigm and from day 1 for the prevention paradigm) and plotted for each group and compared by one-way ANOVA. All data are represented as mean +/- SEM with P < .05 considered significant. The sample size and sex are noted in the figures and figure legends.

Results

Systemic Administration of RmMeteorin Reverses Hindpaw Mechanical Hypersensitivity in Paclitaxel Treated Female and Male Mice

Meteorin produces a robust reversal of neuropathic mechanical hypersensitivity in various rat models of neuropathic pain.
Systemic administration of rmMeteorin reverses hindpaw mechanical hypersensitivity in paclitaxel treated female and male mice. (A) A cohort of male and female mice were administered an i.p. injection of 4 mg/kg paclitaxel every other day for a cumulative dose of 16 mg/kg. This was followed by 5 s.c. injections of 0.5 mg/kg or 1.8 mg/kg of rmMeteorin or Vehicle. Hind paw mechanical thresholds were measured as shown in the figure. (B) Mechanical paw withdrawal thresholds in female groups injected with rmMeteorin (0.5 mg/kg and 1.8 mg/kg) displayed reduced mechanical hypersensitivity compared to the paclitaxel (Ptx) + Vehicle group. N = 8/group until day 23, N = 4/group until day 54 (Two-way ANOVA-mixed effect analysis, F = 4.41, P-value < .0001, post hoc Tukey) (C) Effect size was determined by calculating the cumulative difference between the value for each time point and the baseline value and summed from the beginning of rmMeteorin administration. The effect size difference was significant in the female cohort of mice (Effect size, One-way ANOVA, F = 18.91, P-value = < .0001, post hoc Tukey, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = < .0001). (D) rmMeteorin (0.5 mg/kg and 1.8 mg/kg) administration reversed mechanical withdrawal thresholds in male mice, N = 7 to 8 until day 21 and N = 3 to 4 on day 51 (Two-way ANOVA mixed effect analysis, F = 3.35, P-value < .0001, post hoc Tukey) (E) Effect size difference in male cohorts of reversal paradigm was significant for both MTRN doses, (One-way ANOVA, F = 6.79, P-value = .006, post hoc Tukey, Ptx + Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .018, Ptx + Vehicle vs Ptx + 1.8 mg/kg MTRN, P-value = .0089). Data represents mean +/- SEM. Significance represented as * for Ptx + Vehicle versus 0.5 mg/kg MTRN and # for Ptx + Vehicle versus 1.8 mg/kg MTRN.  

Figure 1. Systemic administration of rmMeteorin reverses hindpaw mechanical hypersensitivity in paclitaxel treated female and male mice. (A) A cohort of male and female mice were administered an i.p. injection of 4 mg/kg paclitaxel every other day for a cumulative dose of 16 mg/kg. This was followed by 5 s.c. injections of 0.5 mg/kg or 1.8 mg/kg of rmMeteorin or Vehicle. Hind paw mechanical thresholds were measured as shown in the figure. (B) Mechanical paw withdrawal thresholds in female groups injected with rmMeteorin (0.5 mg/kg and 1.8 mg/kg) displayed reduced mechanical hypersensitivity compared to the paclitaxel (Ptx) + Vehicle group. N = 8/group until day 23, N = 4/group until day 54 (Two-way ANOVA-mixed effect analysis, F = 4.41, P-value < .0001, post hoc Tukey) (C) Effect size was determined by calculating the cumulative difference between the value for each time point and the baseline value and summed from the beginning of rmMeteorin administration. The effect size difference was significant in the female cohort of mice (Effect size, One-way ANOVA, F = 18.91, P-value = < .0001, post hoc Tukey, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = < .0001). (D) rmMeteorin (0.5 mg/kg and 1.8 mg/kg) administration reversed mechanical withdrawal thresholds in male mice, N = 7 to 8 until day 21 and N = 3 to 4 on day 51 (Two-way ANOVA mixed effect analysis, F = 3.35, P-value < .0001, post hoc Tukey) (E) Effect size difference in male cohorts of reversal paradigm was significant for both MTRN doses, (One-way ANOVA, F = 6.79, P-value = .006, post hoc Tukey, Ptx + Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .018, Ptx + Vehicle vs Ptx + 1.8 mg/kg MTRN, P-value = .0089). Data represents mean +/- SEM. Significance represented as * for Ptx + Vehicle versus 0.5 mg/kg MTRN and # for Ptx + Vehicle versus 1.8 mg/kg MTRN.
trauma-induced peripheral nerve injury but it does not have an effect on normal mechanical thresholds. Accordingly, we did not include a Vehicle + rmMeteorin group in the current study. We sought to investigate the potential antinociceptive effects of rmMeteorin in paclitaxel-induced peripheral neuropathy in male and female mice. We induced neuropathic pain by administering paclitaxel at 4 mg/kg every other day for 4 days. This was followed by 5 s.c. injections of rmMeteorin at .5 mg/kg or 1.8 mg/kg dosage or Vehicle (DPBS) given every-other-day for a total of 10 days (Fig 1A). Administration of paclitaxel induced mechanical hypersensitivity in all groups and both sexes of mice by day 9. Thereafter, administration of both doses of rmMeteorin (.5 mg/kg or 1.8 mg/kg) produced a robust and sustained reversal of mechanical hypersensitivity in male and female mice (Fig 1B, C). Mechanical paw withdrawal thresholds were tested until all animals in all groups returned to baseline. Effect size was significantly different from Vehicle for both doses of rmMeteorin (.5 mg/kg and 1.8 mg/kg) demonstrating efficacy in both sexes (Fig 1D, E).

Systemic Administration of rmMeteorin Reverses Satellite Cell Gliosis Caused by Paclitaxel Treatment

Gliosis of satellite glial cells occurs after chemotherapy treatment causing the cells to change their morphology and release mediators that may contribute to neuronal hyperexcitability associated with neuropathic pain. Therefore, we examined changes in satellite glial cell reactivity within the DRG of the treated cohorts. We assessed immunoreactivity for glutamine synthetase, which labels satellite glial cells that surround the neuronal cell bodies. We did not include a Vehicle treated, baseline group because previous work demonstrates clear satellite cell gliosis in the paclitaxel model. Paclitaxel administration was associated with strong expression of glutamine synthetase in both male and female mice (Fig 2A, B). Administration of rmMeteorin (.5 mg/kg) in the reversal paradigm significantly reduced the expression of glutamine synthetase in both sexes. The 1.8 mg/kg dosage of rmMeteorin also reduced glutamine synthetase expression significantly, albeit only in males (Fig 2C, D). It is unlikely that this represents a true sex difference because 2-way ANOVA analysis did not reveal a significant effect of sex.

Recombinant Mouse Meteorin Reduces Connexin 43 Gap Junction Expression in Mice Treated With Paclitaxel

Satellite cell gliosis caused by chemotherapy is also associated with an increase in neuronal coupling and expression of gap junctions within DRGs. We assessed connexin 43 immunolabeling in DRGs of mice treated with paclitaxel with or without rmMeteorin treatment. We observed clear connexin 43 expression around neurons in paclitaxel treated groups in both sexes. Administration of rmMeteorin (.5 mg/kg) in the reversal paradigm significantly reduced the expression of connexin 43 in both males and females. In contrast, administration of 1.8 mg/kg rmMeteorin had a significant effect only in males (Fig 3C, D).

Recombinant Mouse Meteorin Blocks Development of Hind Paw Mechanical Hypersensitivity in the Prevention Dosing Paradigm

Having shown that rmMeteorin could markedly reverse mechanical hypersensitivity when administered after paclitaxel treatment, we sought to explore whether rmMeteorin could block the development of mechanical hypersensitivity in paclitaxel-induced neuropathy using the prevention dosing paradigm in female mice. We used female mice for these experiments because 1) similar effects were seen in both sexes in the interventive experiment and 2) paclitaxel is reported to cause toxicities more frequently in women. Accordingly, we injected s.c. rmMeteorin .5 mg/kg, 1.8 mg/kg or Vehicle starting 1 day before and then on days between paclitaxel treatments (Fig 4A). Here, we observed that both doses of rmMeteorin attenuated the development of paclitaxel induced mechanical hypersensitivity (Fig 4B). Again, the effect of rmMeteorin was both robust and sustained, with the effect size significantly different from Vehicle for both doses (Fig 4C).

Recombinant Mouse Meteorin Reduces Satellite Cell Gliosis and Gap Junction Expression in the Prevention Dosing Paradigm in Paclitaxel Treated Mice

We assessed whether administration of rmMeteorin attenuated satellite cell gliosis in the prevention dosing paradigm. We observed expression of glutamine synthetase in the paclitaxel treated cohort and this effect was significantly blocked in both groups treated with rmMeteorin (.5 mg/kg and 1.8 mg/kg) (Fig 5A-C). Next, we examined the expression of connexin 43 in the DRG in mice treated with the prevention dosing paradigm. We observed a significant reduction in connexin 43 expression in animals treated with both doses of rmMeteorin (.5 mg/kg or 1.8 mg/kg) compared to the paclitaxel treated cohort alone (Fig 5D,E).

Recombinant Mouse Meteorin Partially Reverses IENF Density Loss Caused by Paclitaxel

Loss of IENFs is a characteristic sign of chemotherapy-induced neuropathy. Paclitaxel treatment is known to cause the retraction of nerve fibers from the epidermis in animals and in patients. We assessed the density of IENFs using PGP9.5 immunostaining. Paclitaxel treatment induced significant loss of IENF in the skin...
Figure 2. rmMeteorin treatment reverses the satellite cell gliosis in the reversal dosing paradigm of paclitaxel treated mice. (A) Representative images of the female cohort show the expression of glutamine synthetase expression in satellite glial cells in green, peripherin (purple) to label neurons, DAPI (blue) to label nuclei. (B) Representative images of satellite glial cells at higher magnification show reduced expression of glutamine synthetase (GS in the figure) in Ptx + 0.5 mg/kg MTRN and Ptx + 1.8 mg/kg MTRN groups compared to Ptx + Vehicle. (C-D) 0.5 mg/kg MTRN (royal blue) and 1.8 mg/kg MTRN (navy blue) reverses satellite cell gliosis expression compared to Ptx + Vehicle (yellow) in both male and female cohorts of mice in the reversal paradigm (females, One-way ANOVA F = 5.78, P-value = .040, post hoc Tukey, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .0465, males, One-way ANOVA, F = 12.97, P-value = .0066, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .022, Ptx + Vehicle vs Ptx + 1.8 mg/kg MTRN, P-value = .0068). N = 3/group. Data are represented as mean ± SEMs. Scale bar = 100 μm (A), and 20 μm (B).
Figure 3. *rmMeteorin reduces gap junction expression in mice treated with paclitaxel.* (A) Representative 20X images of gap junction expression in the DRG showing connexin 43 in male mice (white), peripherin (purple) DAPI (blue). (B) Representative 40X overlay images of gap junction expression in DRG of mice using connexin 43 (white) with peripherin (purple) in the reversal paradigm groups Ptx + Vehicle, Ptx + 0.5 mg/kg MTRN, Ptx + 1.8 mg/kg MTRN. (C) Connexin 43 expression in the DRG was significantly reduced in animals treated with rmMeteorin compared to Ptx + vehicle treated mice in both females and males (Females, One-way ANOVA, F = 7.17, P-value = .026, post hoc Tukey, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .025, Ptx + Vehicle vs Ptx + 1.8 mg/kg MTRN, P-value > .05, Males, One-way ANOVA, F = 14.95, P-value = .00047, Vehicle vs Ptx + 0.5 mg/kg MTRN, P-value = .0088, Ptx + Vehicle vs Ptx + 1.8 mg/kg MTRN, P-value = .0067) N = 3/group. Data are represented as mean ± SEMs. Scale bar = 20 μm.
compared to naïve mice. Administration of both doses of rmMeteorin (.5 mg/kg or 1.8 mg/kg) in the prevention dosing paradigm partially reversed the IENF loss caused by paclitaxel (Fig 6 A,B).

Discussion

In this study, we demonstrated that systemic administration of rmMeteorin results in the resolution of mechanical hypersensitivity in paclitaxel-induced peripheral neuropathy in male and female mice. Our findings align with the previous demonstration that rmMeteorin promotes an antinociceptive effect in peripheral neuropathy models where the neuropathic pain is caused by traumatic injury to peripheral nerves.16,47 Our findings also demonstrate that rmMeteorin treatment reduces 2 signs of satellite cell gliosis, reducing the expression of both glutamine synthetase and the gap junction protein connexin 43. Finally, rmMeteorin partially prevented the retraction of IENFs from the skin of mice treated with paclitaxel. Collectively, these experiments demonstrate that rmMeteorin has a beneficial effect on multiple aspects of paclitaxel-induced neuropathy including improved behavioral and molecular and cellular outcomes.

Recently, KIT receptor tyrosine kinase has been identified in cardiac tissue as a putative receptor for the sibling protein Meteorin-like.38 Although, the receptor-mediated mechanism of action of Meteorin remains unknown, during development Meteorin is known to play an important role in differentiation of glial cells, including satellite glial cells in the DRG.32 Reactive gliosis is characterized by morphological and molecular changes in peripheral and/or central glial cells and is caused by injury to peripheral nerves by trauma or by chemotherapeutic treatment.15,23,32 Satellite cell and astrocytic gliosis results in upregulation of glial fibrillary acidic protein and glutamine synthetase, cellular
Figure 5. rmMeteorin reduces satellite cell gliosis and gap junction expression in the prevention dosing paradigm of paclitaxel treated mice. (A–B) Representative image of satellite cell gliosis measured using glutamine synthetase (GS in the figure) immunoreactivity, peripherin (purple), and DAPI (blue). rmMeteorin reduced satellite cell gliosis in the DRG from the female cohort. C) Significant reduction in glutamine synthetase immunolabelling was observed in mice administered .5 mg/kg (royal blue) and 1.8 mg/kg (navy blue) MTRN in the prevention dosing paradigm compared to paclitaxel + Vehicle (yellow) treated mice (One-way ANOVA, Sankaranarayanan et al. The Journal of Pain 9).
hypertrophy, and proliferation. In the adult DRG, reactive gliosis caused by Transforming Growth Factor-β1 (TGF-β1) triggers the production of endogenous Meteorin, resulting in a negative feedback loop promoting resolution of gliosis. The existing literature suggests a clear role for Meteorin-mediated effects on satellite glial cells but developmental signaling effects may be quite different than those observed on adult cells, which is common for growth factors. Our results show that administration of rmMeteorin reversed satellite cell gliosis after paclitaxel treatment in both reversal and prevention dosing paradigms. While more work is needed to understand the underlying mechanism, our findings support a potential role for Meteorin in disease modifying effects with respect to satellite glial cells in paclitaxel-induced neuropathy.

Satellite glial cells tightly envelop neurons in the DRG. Gap junction-mediated coupling between satellite glial cells is increased after administration of chemotherapy drugs such as paclitaxel and oxaliplatin and this coupling potentially contributes to pain in CIPN. Our results are consistent with the existing literature where paclitaxel induces an increase in the expression of connexin 43 in rodent DRG in satellite glial cells. In our experiments, administration of rmMeteorin reduced the expression of connexin 43 in mouse

Figure 6. Systemic administration of rmMeteorin partially reverses IENF density loss caused by paclitaxel in the prevention dosing paradigm. (A) Representative image of skin from hind paw immunolabeled for fibers with PGP9.5 and nuclei with DAPI (blue). (B) rmMeteorin administration (.5 mg/kg and 1.8 mg/kg) partially reversed the IENF density loss caused by paclitaxel. Ptx + .5 mg/kg MTRN (royal blue) and 1.8 mg/kg MTRN (navy blue) showed significant increased nerve fiber density compared to the Ptx + Vehicle group (One-way ANOVA, F = 29.68, P-value < .0001, post hoc Tukey, Naive vs Ptx + Vehicle, P-value < .0001, naive vs Ptx + .5 mg/kg MTRN, P-value = .0010, naive vs Ptx + 1.8 mg/kg MTRN, P-value = .0021, Ptx + Vehicle vs Ptx + .5 mg/kg MTRN, P-value = .006, Ptx + Vehicle vs Ptx + 1.8 mg/kg, P-value = .0025) N = 3/group. Data are represented as mean ± SEMs. Scale bar = 20 μm.
including nociceptors.32 Meteorin treatment also projected neurons of the developing sensory ganglion, extensive neurite growth in small-and intermediate orin.

likely related to the small sample size and not to an actual sex difference in the biological action of Meteorin. The reason for this small difference at the higher dose of rmMeteorin in the reversal dosing paradigm is not clear at this time but is related to the small sample size and not to an actual sex difference in the biological action of Meteorin.

Previous studies have shown that Meteorin causes extensive neurite growth in small-and intermediate sized neurons of the developing sensory ganglion, including nociceptors.32 Meteorin treatment also promotes neurite outgrowth from DRG explants of neonatal animals.32 This could suggest that in addition to an effect on satellite glial cells, Meteorin may also have a direct action on DRG neurons. Consistent with preclinical and clinical observations with paclitaxel, we observed a loss in IENF density in the skin following chemotherapy treatment. This IENF loss was partially reversed in the prevention dosing paradigm by rmMeteorin at both doses. A shortcoming of this experiment is that it was done only in females, but the greater impact of paclitaxel neurotoxicity on females justifies this choice35 While we favor the hypothesis that rmMeteorin acts directly on nerve endings to protect them from paclitaxel toxicity, it is also possible that Meteorin acts on glial cells surrounding the neuron, to promote breakdown of neurotoxic metabolites, and releases neuroprotective factors that promote the survival of neuronal endings in the epidermis.32,43

There are some limitations to our study. Notably, the complete mechanism of action through which rmMeteorin reduces paclitaxel-induced peripheral neuropathy is not resolved by our work. This will be a primary goal of future studies. Moreover, this study and others in the literature16,47 have only assessed the effect of Meteorin in rodent models and on rodent cells. Bulk, spatial and single-cell sequencing experiments suggest that the METRN gene is highly expressed by neurons and likely glial cells in human DRG, consistent with similar experiments in mice.37,43,48 A key future direction will be to conduct validation studies in human DRG using recombinant human Meteorin to create a compelling rationale for further clinical development. Moreover, we did not include Vehicle treated animals with and without rmMeteorin treatment in von Frey or satellite cell gliosis or IENF analysis. We justified these remarks in the methods and results but acknowledge that the lack of these groups does limit the interpretation of the degree to which rmMeteorin treatment may revert changes back to baseline levels seen in naïve or Vehicle treated mice. Finally, as noted above, we did not conduct all studies in both sexes, but we believe that the findings from the reversal dosing paradigm strongly suggest that consistent effects should be observed. Examining potential differences in Meteorin signaling on male and female human DRG cells will be a critical step to understanding the future potential of this therapeutic approach in clinic.

Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.jpain.2022.10.015.

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