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Evidence that spinal astrocytes but not microglia contribute to the pathogenesis of paclitaxel-induced painful neuropathy

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Abstract

Paclitaxel often induces persistent painful neuropathy as its most common treatment limiting side effect. Little is known concerning the underlying mechanisms. Given the prominent role of glial cells in many types of neuropathic pain, we investigated here the morphological and functional changes of spinal astrocytes and microglia in a rat model of paclitaxel-induced neuropathy. Immunohistochemistry, western blotting and real-time polymerase chain reaction (rt-PCR) were performed with samples from 109 rats up to 28 days after paclitaxel treatment. Paclitaxel (2mg/kg, i.p.) induced a rapid and persistent activation of spinal astrocytes assessed using glial fibrillary acidic protein (GFAP), but not apparent activation of microglia assessed using OX42, Iba-1 and phosphorylated p38. In the context of astrocyte activation, there was a significant downregulation of glial glutamate transporters GLAST and GLT-1 in spinal dorsal horn. The activation of spinal astrocytes by paclitaxel was not associated with expression of pro-inflammatory cytokines including tumor necrosis factor- α (TNF α), interleukin-1 β (IL-1 β) or interleukin-6 (IL-6) in spinal dorsal horn. Systemic treatment with minocycline (50mg/kg, i.p.) prevented activation of astrocytes and downregulation of glial glutamate transporters in spinal dorsal horn induced by paclitaxel. These data suggest the involvement of spinal astrocytes but not microglia in the pathogenesis of paclitaxel-induced neuropathy.

Keywords

Paclitaxel; neuropathy; spinal cord; astrocyte; microglia; minocycline

INTRODUCTION

Paclitaxel (Taxol®) is one of the most effect chemotherapeutic drugs widely used for the treatment of solid tumors including ovarian, breast and non-small cell lung cancer. The use of paclitaxel is often associated with neurotoxicity that predominantly results in sensory dysfunction and pain^{8,1}. An acute pain syndrome associated with paclitaxel peaks as early as three days after chemotherapy²⁶, while chronic neuropathy occurs between the first and fourth cycle of treatment and can last from months to years¹⁰. Paclitaxel-induced neuropathy

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DISCLOSURES

All authors declare no conflict of interest.

is one of the major dose-limiting factors and can significantly impact on rehabilitation and the return to productivity of thousands of cancer survivors. Unfortunately, the mechanism of the paclitaxel-induced chronic pain is not well understood.

Paclitaxel-induced neuropathy has been successfully modeled in experimental animals by both single high dose and intermittent repeated low dose injections^{33,34,9}. Although a number of studies are in the literature regarding the peripheral mechanisms of paclitaxel-induced neurotoxicity including axonopathy and ganglionopathy¹, the central mechanisms of paclitaxel-induced chronic pain have been less well studied. Previous studies in our lab using in vivo electrophysiological recording on spinal dorsal horn neurons have shown that spinal dorsal horn neurons display enhanced activities after paclitaxel treatment which suggests a paclitaxel-induced central plasticity⁵. It has been demonstrated recently that spinal astrocytes and microglia play critical roles in facilitating central plasticity following both nerve injury and inflammation^{27,36}. The functional status of spinal astrocytes and microglia following paclitaxel has been briefly studied but the results are somewhat controversial. The activation of spinal astrocytes was found with high-dose (cumulative dose 36mg/kg)³³ but not with low-dose (cumulative dose 8mg/kg)²³ paclitaxel treatment. The previous finding that paclitaxel induced activation of spinal microglia^{33,23} has also been challenged by a recent study that no activation of spinal microglia was observed after the same treatment of paclitaxel by probing a specific microglia marker Iba-1⁵⁵. In the present study, we investigated the functional status of spinal astrocytes and microglia following paclitaxel treatment as long as 28 days after treatment and explored how spinal glial cells contribute to the development of paclitaxel-induced painful neuropathy in a rat model^{5,6,4}.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (8–10 weeks, Harlan, Houston, TX, USA) housed in a 12 h light/dark cycle with free access to food and water were used in all experiments. 109 rats in total were used in the study. The studies were approved by the Institutional Animal Care and Use Committee at The University of Texas M. D. Anderson Cancer Center and were performed in accordance with the National Institutes of Health Guidelines for Use and Care of Laboratory Animals. Maximum efforts were made to minimize the number of animals and any discomfort involved in all procedures.

Paclitaxel-induced neuropathy model

Animals were treated with paclitaxel as previously described⁴. Briefly, paclitaxel (TEVA Pharmaceuticals, Inc. USA) was diluted with sterile saline from the original concentration of 6 mg/ml (in Cremophor EL/ethanol 1:1) to 1 mg/ml and given at a dosage of 2 mg/kg (intraperitoneally) every other day for a total of four injections (Day 1, 3, 5, and 7). Control animals received an equivalent volume of vehicle (Cremophor EL/ethanol 1:1). Rats were observed carefully for any abnormal behavioral changes twice a week during and after paclitaxel or vehicle treatment.

Spinal nerve ligation

The L5 spinal nerve was ligated as described by Kim and Chung²². Briefly, rats were anesthetized with isoflurane and the ipsilateral L6 transverse process was removed using aseptic surgical technique. The L5 spinal nerve was then ligated with 5-0 silk. Control rats underwent a sham surgery wherein the L5 spinal nerve was exposed but not ligated. Muscle, fascia and skin were sequentially closed in layers. All rats were allowed to recover for three to five days after surgery before subject to other procedures.

Minocycline treatment

Minocycline hydrochloride (Sigma, St. Louis, MO, USA) was diluted in saline and buffered to a pH of 7.0 with NaOH and given intraperitoneally at a dose of 50mg/kg starting 72 hours prior to the first injection of paclitaxel. Minocycline was continued every day for the next 3 days for a total of 4 injections. On days when both drugs were administered, minocycline was given 30 minutes prior to paclitaxel.

Immunohistochemistry

Animals were deeply anesthetized with sodium pentobarbital (Nembutal, 100 mg/kg, i.p.) and perfused through the ascending aorta with warm saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer. Spinal cord segments L4-5 were removed and postfixed in 4% paraformaldehyde and then cryoprotected in 30% sucrose solution at 4°C. Transverse spinal cord sections (25 μ m) were cut in a cryostat and processed for immunofluorescent staining. Every fourth spinal cord section from each sample was collected to probe the same protein. Spinal cord sections were first blocked with 10% normal donkey serum (NDS) and 0.2% Triton X-100 in PBS for 1 hr at room temperature. The sections were then incubated over night at 4°C in 5% NDS and 0.2% Triton X-100 in PBS containing the primary antibodies for the following targets: GFAP (mouse, 1:1000, Cell Signaling Technology), OX-42 (mouse, 1:1000, Serotec), GLT-1 (guinea pig, 1:3000, Millipore), and GLAST (Guinea pig, 1:3000, Millipore). After washing, the sections were then incubated with Cy3- or FITC-conjugated secondary antibodies over night at 4°C. Sections were then mounted on glass slides and were viewed under a fluorescent microscope (Eclipse E600, Nikon, Japan). The quantification of immunofluorescent staining was performed as described before^{52,51}. For a given experiment, all images were taken using identical acquisition parameters and the final representative figures are presented as the original images without further modification. To quantify the intensity of immunohistochemical staining in spinal lamina II, images were captured under 10X or 20X objectives and the area of spinal lamina II of each section was determined when viewed under differential interference contrast (DIC) (Fig. 1A, B). Background fluorescence was measured first by defining 3 – 5 circular regions in the spinal lamina II adjacent to immune-positive neurons and averaged to obtain a mean background value for each section (Fig. 1C). The mean fluorescent intensity subtracted by background for the whole of spinal lamina II of each section was determined. All images were analyzed using NIC Elements imaging software (Nikon, Japan). At least five sections were quantified for each animal.

Western blot

The L4–L5 spinal dorsal horn were collected from animals deeply anesthetized with pentobarbital sodium (Nembutal, 100 mg/kg, i.p.) and snap-frozen in liquid nitrogen. Tissues were later homogenized in RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄ and 1 μ g/ml leupeptin) mixed with protease inhibitor cocktail (P8340, Sigma) and phosphatase inhibitor cocktails (P0044 and P5726, Sigma) on ice. The supernatant was then collected and denatured with sample buffer ($\times$ 5) consisting of 0.25 M Tris-HCl, 52% glycerol, 6% SDS, 5% β -Mercaptoethanol and 0.1% bromophenol blue for 5 minutes at 95°C. Samples were separated by electrophoresis using Novex 4–20% Tris-Glycine gel with total protein of 25 μ g and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad). After blocking with 5% low-fat milk in TBS containing 0.1% Tween 20 (TBST) for 1 hr at room temperature, PVDF membranes were probed with primary antibodies overnight at 4°C. After washing, membranes were then probed with secondary antibodies labeled with horseradish peroxidase (Calbiochem, CA, USA) for 1 hr at room temperature and detected with chemiluminescent reagents. The PVDF membranes were stripped with the Restore® western blot stripping

buffer (Thermo Scientific, Rockford, IL, USA) and re-probed with antibody to β -actin. The primary antibodies used in the experiment included: phospho-p38 (rabbit, 1:2000, Cell Signaling Technology), Iba-1 (mouse, 1:1000, Dako, Japan), GLT-1 (guinea pig, 1:3000, Millipore), GLAST (guinea pig, 1:3000, Millipore) and β -actin (1:10,000; Sigma). Immunopositive bands were detected by Amersham® ECL or ECL plus (GE Healthcare, little Chalfont, UK) and were quantified with Image J (NIH).

Real-time polymerase chain reaction (rt-PCR)

The tissues for real-time PCR were collected as mentioned in the western blot experiments. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. The concentration and purity of RNA were detected by measuring the absorbance at 260 and 280 nm in a spectrophotometer (Spectra Max Plus 384, Molecular Device, CA, USA). Reverse transcription of cDNA from 1 μ g of total RNA was performed in a thermal cycler (GeneAmp PCR System 9700, Life Technologies, Carlsbad, CA, USA) by Superscript III First-Strand Synthesis SuperMix (Invitrogen). The reactions were performed with a mixture of sample cDNA, oligo(dT)₂₀, Annealing Buffer, 2 $\times$ First-Strand Reaction Mix and SuperScriptIII/RNaseOUT Enzyme Mix in a total volume of 20 μ l. Control reactions lacking either reverse transcriptase or template were included to assess carryover of genomic DNA and non-specific contamination, respectively. The amplification of cDNA was performed using SYBR Green PCR Master Mix (ABI, Warrington, UK) on a 7000 Sequence Detection system (Life Technologies, Carlsbad, CA, USA). Primers used in the experiment included: TNF α (D00475): 5'-CTTCAAGGGACAAGGCTG-3' (F), 5'-GAGGCTGACTTTCTCCTG-3' (R); IL-1 β (M98820): 5'-GAAGTCAAGACCAAGTGG-3' (F), 5'-TGAAGTCAACTATGTCCCG-3' (R); IL-6 (NC005013): 5'-AAGTTTCTCTCCGCAAGAGACTTCCAG-3' (F), 5'-AGGCAAATTTCTGTTATATCCAGTT-3' (R); glyceraldehyde-3-phosphate-dihydrogenase (GAPDH, NM01008): 5'-TGCCAAGTATGATGACATCAAGAAG-3' (F), 5'-AGCCCAGGATGCCCTTTAGT-3' (R). Amplification steps consisted of one cycle of 50°C for 2 minutes plus 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 55°C for 25 seconds and 72°C for 1 minute, and one cycle of 95°C for 15 seconds, 60°C for 20 seconds and 95°C for 15 seconds as a dissociation stage. The threshold cycle (C_t ; the number of cycles to reach the threshold of detection) was determined for each gene and the relative expression level of each gene was calculated using the following formula: relative expression of mRNA = $2^{-(\Delta C_{t\text{sample}} - \Delta C_{t\text{control}})}$, where $C_{t\text{sample}}$ is the C_t for the target gene and $C_{t\text{control}}$ is the C_t for the housekeeping gene GAPDH³⁸.

Statistical analysis

All results are presented as means $\pm$ SEM and analyzed with Sigma Plot 10.0 (Systat Software, Inc., San Jose, CA, USA). Differences between means were tested for significance using paired t -test, one-way (with Student Newman-Keuls post hoc test) or two-way ANOVA (with Bonferroni post hoc test) with an alpha value of $P < 0.05$.

RESULTS

Paclitaxel induces activation of spinal astrocytes

The activation of spinal astrocytes was evaluated by the expression of GFAP. As shown in Figure 2, systemic treatment of paclitaxel induced a significant increase in GFAP expression in spinal dorsal horn as early as 4 hours after the first injection compared to vehicle-treated animals ($p < 0.01$). The level of GFAP remained significantly elevated to 28 days after paclitaxel treatment, the last time point we observed (time $F(1,38) = 67.95$, $P < 0.0001$; treatment $F(5,38) = 3.52$, $P = 0.0104$; interaction $F(5,38) = 0.8$, $P = 0.56$). The levels of GFAP in the vehicle-treated group remained consistently low throughout the observation

time (Fig. 2). Although the expression of GFAP was only quantified in spinal dorsal horn, the increase of GFAP was found throughout the layers of spinal cord grey matter including areas surrounding central canal (Fig. 2A) and ventral horn (data not shown).

Paclitaxel does not induce the activation of spinal microglia

The activation of spinal microglia was first evaluated by the expression of a cell surface marker for macrophages, CD11b (also called OX42 in rat). OX42 has been used to study the activation of microglia in various rat models of chronic pain including SNL^{36, 2}. As shown in Figure 3A, SNL induced a robust increase in OX42 expression in ipsilateral spinal dorsal horn compared to the contralateral side ($n=4$, $P < 0.01$, ipsilateral vs. contralateral) (Fig. 3A, C). In contrast, there was no increase in OX42 expression in spinal dorsal horn at any of the time points we observed (4 hours, 7 days and 28 days) after paclitaxel treatment (Fig. 3B, C).

To further evaluate the functional status of spinal microglia, two more markers of activated microglia: Iba-1 and p-p38 MAPK, were probed. Both markers have been demonstrated to be expressed exclusively in activated microglia in rats^{20,21,56,37,42}. As shown in Figure 4A, SNL induced a significant increase of Iba-1 in spinal dorsal horn compared to sham-operated or naïve rats ($F(2,8) = 107.1$, $P < 0.0001$). In contrast, no increase in Iba-1 was observed after paclitaxel treatment (Fig. 4B). Similarly, a significant increase of p-p38 was induced in spinal dorsal horn by SNL ($F(2,8) = 102.5$, $P < 0.0001$, Fig. 4C) but not paclitaxel treatment (Fig. 4D).

Paclitaxel induces the downregulation of spinal glial glutamate transporters

In spinal cord, GLAST and GLT-1 are expressed predominantly in astrocytes⁴⁹. Our previous studies have shown that paclitaxel treatment induces the downregulation of both GLAST and GLT-1^{5,46}, but their contribution to paclitaxel-induced mechanical hypersensitivity is not clear. Here the expression of GLAST and GLT-1 was evaluated in detail by western blotting following paclitaxel treatment. As shown in Figure 5, paclitaxel induced a rapid and significant downregulation of GLAST in spinal dorsal horn as early as 4 hours after treatment compared to vehicle-treated or naïve animals (treatment $F(2,32) = 32.12$, $P < 0.0001$; time $F(3,32) = 2.11$, $P = 0.12$; interaction $F(6,32) = 2.44$, $P = 0.047$). The level of GLAST remained significantly decreased through 16 days after paclitaxel treatment (Fig. 5A, B). The downregulation of GLT-1 in spinal dorsal horn was only observed at 4 hours after paclitaxel treatment (treatment $F(2,32) = 3.0$, $P = 0.064$; time $F(3,32) = 5.12$, $P = 0.0053$, interaction $F(6,32) = 3.88$, $P = 0.005$) and then recovered at 7 days after treatment (Fig. 5C, D). In contrast, vehicle treatment did not induce any change in the expression of either GLAST or GLT-1 compared to naïve animals (Fig. 5A–D).

Paclitaxel does not induce the expression of pro-inflammatory cytokines in spinal dorsal horn

Recent studies have shown that activated astrocytes often release pro-inflammatory cytokines such as TNF α , IL-1 β and IL-6 and contribute to the maintenance of neuropathic or inflammatory pain^{18,45,28,32}. To examine if pro-inflammatory cytokines were released by activated spinal astrocyte following paclitaxel treatment, the expression of TNF α , IL-1 β and IL-6 was quantified by real-time PCR. Samples were examined at both 4 hours and 7 days after treatment. As shown in Figure 5E, there was no significant increase in the expression of TNF α , IL-1 β or IL-6 in spinal dorsal horn at either time point after paclitaxel treatment.

Minocycline prevents paclitaxel-induced activation of astrocytes and downregulation of glial glutamate transporters in spinal dorsal horn

We have demonstrated that minocycline prevents paclitaxel-induced mechanical hypersensitivity and the loss of intraepidermal nerve fibers^{6,4}. Yet the mechanisms of the preventive effect of minocycline on paclitaxel-induced hypersensitivity are not clear. Here we tested to see if the preventive effect of minocycline included inhibition of the reactions of spinal astrocytes to chemotherapy. The comparisons were made from tissues collected 4 hours after paclitaxel or vehicle treatment. As shown in Figure 6, minocycline treatment not only prevented the activation of astrocytes but also prevented the downregulation of both GLAST and GLT-1 in spinal dorsal horn induced by paclitaxel.

DISCUSSION

In recent years, a large body of literature has demonstrated that the central mechanisms of chronic pain following either nerve injury or inflammation involve not only the plasticity of neurons but also the altered functions of glial cells including astrocytes and microglia in spinal cord^{27,36,39}. In present study, we first demonstrated that paclitaxel treatment induced the activation of spinal astrocytes but not microglia. We next showed that the involvement of spinal astrocytes in the pathogenesis of paclitaxel-induced neuropathy was possibly by downregulating glial glutamate transporters GLAST and GLT-1 but not by releasing pro-inflammatory cytokines such as TNF α , IL-1 β and IL-6 in spinal dorsal horn. Furthermore, systemic treatment with minocycline which has been shown to prevent paclitaxel-induced neuropathy also prevented activation of astrocytes and downregulation of both GLAST and GLT-1 in spinal dorsal horn. Our data strongly suggest a critical role of spinal astrocyte but not microglia in the pathogenesis of paclitaxel-induced neuropathy.

The activation of spinal astrocytes was reported with high-dose (cumulative dose 36mg/kg)³³ but not low-dose (cumulative dose 8mg/kg)²³ paclitaxel treatment. We have observed a rapid increase in the expression of GFAP, indicating activation of astrocytes, in spinal dorsal horn following low-dose (8mg/kg) paclitaxel treatment. The activation of spinal astrocytes persisted during our observation, from 4 hours to 28 days following treatment. Since only late time point (35 or 42 days after treatment) was examined in the previous study with the same dose of paclitaxel as we used, it is possible that the level of GFAP in spinal cord has already declined by that time. The differences in methods to detect GFAP in spinal cord (immunofluorescence vs. avidin-biotin method) also need to be considered.

Paclitaxel-induced mechanical hypersensitivity in rats occurs as early as several hours⁹ or 3 to 7 days^{34,5,6} after treatment and can last several weeks^{6,9,34} to several months²³. The rapid and persistent activation of spinal astrocytes parallels the development of paclitaxel-induced mechanical hypersensitivity. This pattern of astrocyte activation is similar to that following peripheral nerve injury or inflammation⁵⁴. Although it is generally believed that paclitaxel does not penetrate blood-brain-barrier^{19,16}, low concentrations of paclitaxel can be detected in spinal cord after systemic treatment⁷. It has been reported that microtubule stabilizers such as paclitaxel can directly change the distribution and expression of intermediate filaments including GFAP in cultured astrocytes^{40,17}. Thus further study is needed to evaluate whether the activation of spinal astrocytes is induced by the direct effect of paclitaxel or through its effects on peripheral targets.

Spinal astrocytes can modulate neuronal activities through various mechanisms^{27,36}. One important facet of astroglial functions contributing to spinal sensory encoding is the ability of clearing transmitters released from presynaptic sites via specific transporters¹². Glutamate is a major excitatory neurotransmitter released from primary afferent terminals and critical for spinal excitatory synaptic transmission. GLAST and GLT-1 are two types of glial

glutamate transporters predominantly expressed on spinal astrocytes to uptake glutamate^{53,25,49}. Blockade of spinal GLAST and GLT-1 induces spontaneous nociceptive behaviors and hypersensitivity to both peripheral thermal and mechanical stimuli in rats²⁵. Enhanced activities of neurons and excessive activation of postsynaptic AMPA and NMDA receptors in spinal dorsal horn can be induced by blocking GLAST and GLT-1^{47,29,30}. Downregulation of GLAST and GLT-1 in spinal dorsal horn induced by peripheral nerve injury facilitates the development of neuropathic pain which is reversed by restoring activity of glial glutamate transporters^{48,41}. We here confirmed the downregulation of GLAST and GLT-1 induced by paclitaxel which is consistent with our previous studies^{5,46}. We further identified that the downregulation of both GLAST and GLT-1 occurred rapidly following paclitaxel treatment and lasted for several weeks (especially GLAST). The early recovery of GLT-1 in the present study is different from our previous observation but this may be due to the higher sensitivity of ELISA used in the previous study than western blot in the present study. Nevertheless, downregulation of glial glutamate transporters induced by paclitaxel could lead to central sensitization and explains well the increased spontaneous activity and increased afterdischarges of spinal neurons to peripheral stimuli following paclitaxel treatment⁵. The specific role of GLAST or GLT-1 alone involved in spinal synaptic transmission will need further study since spinal astrocytes express both glutamate transporters^{25,49} and the GLAST- or GLT-1- mediated transporter current seems comparable⁵³. But since the downregulation of GLAST persists longer than GLT-1 (Fig. 5B, D), it is possible that GLAST is involved in both initiation and maintenance but GLT-1 is only involved in initiation of paclitaxel-induced pain.

Upon activation, spinal astrocytes often release a number of pro-inflammatory cytokines such as TNF α , IL-1 β and IL-6 to facilitate activities of spinal neurons and modulate spinal synaptic transmission^{27,13}. We did not detect any increase of TNF α , IL-1 β or IL-6 in spinal dorsal horn following paclitaxel treatment by rt-PCR, consistent with a previous report that paclitaxel induced increased signals of TNF α and IL-1 β in dorsal root ganglia but not in spinal cord²³.

The activation of spinal microglia following paclitaxel treatment is controversial. Although the upregulation of OX42 has been found in spinal dorsal horn in rats treated with both high-dose³³ and low-dose²³ paclitaxel, a recent study reported that there was no activation of spinal microglia following low-dose paclitaxel treatment by measuring the expression of Iba-1⁵⁵. We carefully examined the activation of spinal microglia by probing three different markers of activated microglia, OX42, Iba-1 and p-p38, at four different time points (4 hours, 7 days, 16 days and 28 days) following low-dose paclitaxel treatment. Meanwhile, SNL – a well-known model to produce a robust activation of spinal microglia – was used as a positive control to warrant effective staining during the experiments. No significant activation of spinal microglia was found at any time point during our observation following paclitaxel treatment, confirming results reported by Zheng et al⁵⁵. Our data does not agree with the previous study that a robust activation of microglia in spinal cord was observed at 35 or 42 days following the same paclitaxel treatment²³. Although we did not examine the activation of microglia beyond 28 days after the treatment, it is unlikely that a late activation of spinal microglia would occur since the recovery of paclitaxel-induced mechanical hypersensitivity usually starts beyond 28 days after low-dose treatment^{34,9}.

Another intriguing finding in our study is that the preemptive treatment of minocycline prevented paclitaxel-induced activation of spinal astrocytes and downregulation of glial glutamate transporters GLAST and GLT-1. Our previous studies have shown the preventive effect of minocycline on paclitaxel-induced mechanical hypersensitivity and loss of intraepidermal nerve fibers^{6,4}. Although minocycline is generally considered as an inhibitor to microglia^{43,50}, it also has inhibitory effect on astrocytes^{35,14,15,11,44}. In addition,

minocycline prevents the excessive activation of postsynaptic NMDA receptors in spinal dorsal horn by preventing the downregulation of glial glutamate transporters after peripheral nerve injury³¹. Our and others' data have shown that minocycline alone does not change the baseline level of GFAP in spinal cord²⁴ and cultured astrocytes¹⁵ or induce any change in animal behaviors^{4,3,6,24}. The inhibitory effect of minocycline we observed could also be due to the suppression on the release of pro-inflammatory cytokines in dorsal root ganglia following paclitaxel treatment²³. Nevertheless, our data suggest the critical contribution of spinal astrocytes to the central mechanisms of paclitaxel-induced neuropathy. Since minocycline has been used in clinic to treat various diseases, it would be very useful to test its potential effect to prevent paclitaxel-induced painful neuropathy.

In summary, we reported different responses of spinal astrocytes and microglia following paclitaxel treatment. Our data suggest the involvement of spinal astrocytes in the pathogenesis of paclitaxel-induced painful neuropathy, presumably through altering spinal synaptic transmissions by downregulating glial glutamate transporters GLAST and GLT-1. Preemptive treatment of minocycline prevented paclitaxel-induced spinal astroglial responses. Thus spinal astrocytes and/or glutamate transporters could be potential targets for the treatment of paclitaxel-induced neuropathy.

PERSPECTIVE

Spinal astrocytes and/or glial glutamate transporters could be new therapeutic targets for paclitaxel-induced painful neuropathy.

Acknowledgments

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Figure 1.

Illustration of quantifying immunohistochemical stainings. A, A spinal dorsal horn was viewed under differential interference contrast (DIC) and the area of spinal lamina II was determined. B, The same slice was viewed under fluorescence without changing the position. C, Background fluorescence was measured first by defining 3 circular regions (1–3) adjacent to immune-positive neurons in spinal lamina II and averaged to obtain a mean background value. Then the mean fluorescent intensity subtracted by background for the whole of spinal lamina II of each section was determined. Scale: 50 μ m.

Figure 2. Expression of GFAP in spinal cord after paclitaxel or vehicle treatment. (A) Representative spinal cord slices of GFAP staining 4 Hrs (a, b), 7 days (c, d) and 28 days (e, f) after treatment. (B) Quantification of GFAP in spinal lamina II in paclitaxel- and vehicle-treated groups. The number of animals in each group was presented in bar graphs. * $p < 0.05$, ** $p < 0.01$ for paclitaxel vs. vehicle, two-way ANOVA with Bonferroni post hoc test. Scale: 500 μm .

Figure 3.

Expression of OX42 in spinal dorsal horn after L5 spinal nerve ligation (SNL) and paclitaxel treatment. (A) A robust increase in the expression of OX42 was detected in ipsilateral (ipsi) spinal dorsal horn versus contralateral (contra) side 7 days after SNL. Lower panels show the enlargement views of ipsilateral and contralateral dorsal horns separately. (B) Paclitaxel did not induce any increase in the expression of OX42 in spinal dorsal horn. (C) Statistical analysis revealed a significant increase in the expression of OX42 induced by L5-SNL but not paclitaxel. N=4 for each group. * $p < 0.01$, ipsilateral vs. contralateral, paired t -test. Scale: 500 μ m.

Figure 4.

Expression of Iba-1 and p-p38 in spinal dorsal horn. (A) Spinal nerve ligation (SNL) induced a significant increase in the expression of Iba-1 in spinal dorsal horn than sham or naïve animals. N=4 for each group. (B) Paclitaxel (Pac) did not induce any increase in the expression of Iba-1 compared to vehicle (Veh) or naïve groups. N=4 for each group. (C) SNL induced a significant increase in the expression of p-p38 in spinal dorsal horn than sham or naïve animals. N=4 for each group. (D) Paclitaxel did not induce any increase in the expression of p-p38 compared to vehicle or naïve groups. N=4 for each group. * $p < 0.05$, ** $p < 0.01$, one-way ANOVA with Student Newman-Keuls post hoc test.

Figure 5.

Expression of GLAST and GLT-1 in spinal dorsal horn. (A, B) Paclitaxel (Pac) but not vehicle (Veh) induced a persistent downregulation of GLAST in spinal dorsal horn. (C,D) Paclitaxel but not vehicle induced a rapid downregulation of GLT-1 in spinal dorsal horn. N=4 for each group. * $p < 0.05$, ** $p < 0.01$ for paclitaxel vs. vehicle; $^{\wedge}$ $p < 0.05$, $^{\wedge\wedge}$ $p < 0.01$ for paclitaxel vs. naïve, two-way ANOVA with Bonferroni post hoc test. (E) Quantification of pro-inflammatory cytokines in spinal dorsal horn by real-time PCR. Paclitaxel did not induce any increase in the expression of TNF α , IL-1 β or IL-6 in spinal dorsal horn compared to vehicle or naïve animals. N=4 for each group.

Figure 6.

Systemic treatment of minocycline prevented the increased expression of GFAP and downregulation of GLAST and GLT-1 in spinal dorsal horn induced by paclitaxel. The number of animals in each group was presented in bar graphs. Veh: vehicle; Pac: paclitaxel; Mino: minocycline. * $p < 0.01$, one-way ANOVA with Student Newman-Keuls post hoc test. Scale: 500 μ m.