Participation of Microglial p38 MAPK in Formalin-Induced Temporomandibular Joint Nociception in Rats

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Aims: *To investigate nociceptive behavior and the immunoreactivity of microglia and phosphorylated-p38 (p-p38) mitogen-activated protein kinase (MAPK) following intracisternal administration of SB203580, a p38 MAPK inhibitor, or minocycline, a microglia inhibitor, in rats with temporomandibular joint (TMJ) inflammation.* ***Methods:*** *The number of nociceptive behavioral responses was recorded for nine successive 5-minute intervals following formalin injections into the left TMJ. SB203580 or minocycline was administered intracisternally 2 hours prior to the formalin injection. Statistical analysis used one-way analysis of variance followed by least significant difference post-hoc analysis.* ***Results:*** *The intra-articular injection of formalin increased the expression of p-p38 MAPK in the ipsilateral medullary dorsal horn. Most of the p-p38 MAPK co-localized with OX42, a microglial marker, but not with GFAP, an astrocyte marker. Intracisternal injections of SB203580 (0.5, 1, or 5 μg) attenuated the number of nociceptive behavioral responses and the expression of p-p38 MAPK in the medullary dorsal horn. Intracisternal injections of minocycline (25 or 50 μg) also attenuated the responses and the expression of OX42 and p-p38 MAPK in the medullary dorsal horn.* ***Conclusion:*** *These findings suggest that p38 MAPK in microglia plays an important role in the central processing of inflammatory TMJ nociception in rats. The data further indicate that a targeted blockade of the microglial p38 MAPK pathway is a potentially important new treatment strategy for inflammatory TMJ nociception.* J OROFAC PAIN 2012;26:132–141

Key words: formalin, microglia, p38 MAPK, pain, TMJ

Peripheral inflammation increases the response to painful stimuli by direct actions of inflammatory mediators, such as interleukin-1β (IL-1β), on primary sensory neurons.[1] Peripheral inflammation also activates glial cells in the central nervous system via neurotransmitters released from the central terminals of activated peripheral nerves. In various inflammatory pain models, subcutaneous irritants or intraperitoneal inflammation have been reported to produce glial activation that parallels enhanced nociceptive responses.[2–4] Moreover, several previous results have demonstrated that neuron-glia crosstalk[5,6] and glia metabolic processes[7,8] play an important role in the development of central sensitization in the medullary dorsal horn. These results suggest that the medullary dorsal horn glial cells have important roles in the induction or maintenance of pain facilitation in animal models of peripheral inflammation in the orofacial area.

Pain in the temporomandibular joint (TMJ) is one of the chief complaints of patients with a TMJ disorder. The underlying mechanisms

of TMJ pain remain poorly understood because of the involvement of multiple factors in the pathogenesis. Modulation of inflammatory hyperalgesia in the TMJ also takes place at the level of the trigeminal brainstem, and glial cells have been implicated in this process.[9,10] However, while the role of glial cells in central nociceptive mechanisms has been well established, very little attention has been given to the involvement of the glia in central mechanisms related to TMJ pain.

The authors have previously utilized the technique of studying nociceptive behaviors induced in rats by the intra-articular injection of formalin into the TMJ.[11,12] The present study investigated the roles of microglia and p38 mitogen-activated protein kinase (MAPK) in TMJ inflammation-induced nociception. Nociceptive behavior was monitored after the intracisternal administration of SB203580, a p38 MAPK inhibitor, or minocycline, a microglia inhibitor, in rats with TMJ inflammation. An immunohistochemical analysis was also carried out to determine the activation of microglia and p38 MAPK expression in the medullary dorsal horn.

Materials and Methods

Animals

Experiments were carried out on 140 male Sprague-Dawley rats weighing 220 to 280 g. The animals were maintained in a temperature-controlled room (23 ± 1°C) with a 12/12 hour light-dark cycle (light on at 7:00 AM). All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee of the School of Dentistry, Kyungpook National University, and were carried out in accordance with the ethical guidelines for the investigation of experimental pain in conscious animals proposed by the International Association for the Study of Pain. All experiments were performed and analyzed in a blind fashion.

Intra-articular Injection of Formalin into the TMJ

Each animal was first placed in a Plexiglas box for 30 minutes to minimize stress. Rats were not allowed access to food or water during the test. After the acclimation period, each animal was removed from the test chamber and anesthetized through the inhalation of 3% isoflurane. Formalin (5%) was then injected with a 30-gauge needle into the capsule of the left TMJ, as described previously.[11–13] The volume of the TMJ injections was set at 50 μL. Following the injections, the animals immediately recovered from the anesthesia and were returned to the test chamber for a 45-minute observation period. For each animal, the number of noxious behavioral responses, such as grooming, rubbing, and/or scratching of the TMJ region, was recorded for nine successive 5-minute intervals. Behavioral responses induced by the formalin injection into the TMJ did not display the usual two distinct phases since the early phase was partly masked by the anesthesia. Hence, the total number of scratches in the second phase (10 to 45 minutes, second phase) was analyzed to provide indices of TMJ nociception after formalin injection, as described previously.[11–13]

To minimize the possibility that the behaviors produced by formalin might have resulted from its effect on regions outside of the TMJ, off-site injections were also performed. The same volume of formalin was injected into the right masseter muscle. In a separate series of experiments, saline was also injected into the TMJ region as a control.

Effects of Intracisternally Injected SB203580 or Minocycline on Formalin-Induced TMJ Nociception

Animals were anesthetized with pentobarbital sodium (40 mg/kg, ip) 3 days prior to formalin injections. The anesthetized rats were then individually mounted on a stereotaxic frame, and a polyethylene (PE10) tube was implanted to enable an intracisternal injection, as described previously.[11,14] The polyethylene tube was subcutaneously led to the top of the skull and secured in place by a stainless-steel screw and dental acrylic resin. After a 72-hour recovery period from the surgery, SB203580 (0.5, 1, or 5 μg/10 μL) or minocycline (25 or 50 μg/10 μL) was administered intracisternally through the implanted PE tube 2 hours prior to the formalin injection in freely moving rats. Because intrathecal catheterization may produce motor dysfunction, only animals that displayed normal motor functions were subsequently evaluated. For confirmation of the placement of the intracisternal cannula and the extent of the spread of the drugs, pontamine sky blue dye was injected at the end of these tests. Animals that showed motor dysfunction or malposition of the catheter after intracisternal catheterization were excluded from the analysis.

Immunofluorescence Staining

At 1 hour after formalin injection into the TMJ, rats (n = 4 per group) were perfused through the ascending aorta with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB,

pH 7.4) under anesthesia. The caudal medullae were dissected out, postfixed in the same fixative at 4°C overnight, and then replaced with 30% sucrose in 0.1 M PB overnight. Transverse frozen sections (free-floating, 30 μm) were prepared using a cryostat and processed for immunofluorescence. All sections (30 μm) were blocked with 5% goat serum in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 for 1 hour at room temperature and then incubated overnight at 4°C with a rabbit polyclonal anti-phosphorylated-p38 (p-p38) MAPK antibody (1:100; Cell Signaling Technology). Following incubation, tissue sections were washed and incubated for 2 hours at room temperature in the Alexa 555-conjugated rabbit IgG antibody (1:200; Invitrogen). For double immunofluorescence, the sections were incubated with a mixture of rabbit anti-p-p38 MAPK and mouse anti-NeuN (neuronal marker, 1:1000; Millipore), mouse anti-OX42 (microglial surface marker, 1:100; Millipore), or mouse anti-GFAP (glial fibrillary acidic protein, 1:1000; Cell Signaling Technology) overnight at 4°C, followed by a mixture of Alexa 555-conjugated rabbit IgG and Alexa 488-conjugated mouse IgG (both 1:200; Invitrogen). For negative controls, the primary antibody was omitted by PBS, but all incubation steps were identical. The stained sections were then observed under a fluorescence microscope (BX 41 and U-RFL-T; Olympus). Co-localization analysis for immunofluorescence images was examined with a confocal laser scanning microscope (LSM 510; Carl Zeiss).

Western Blot

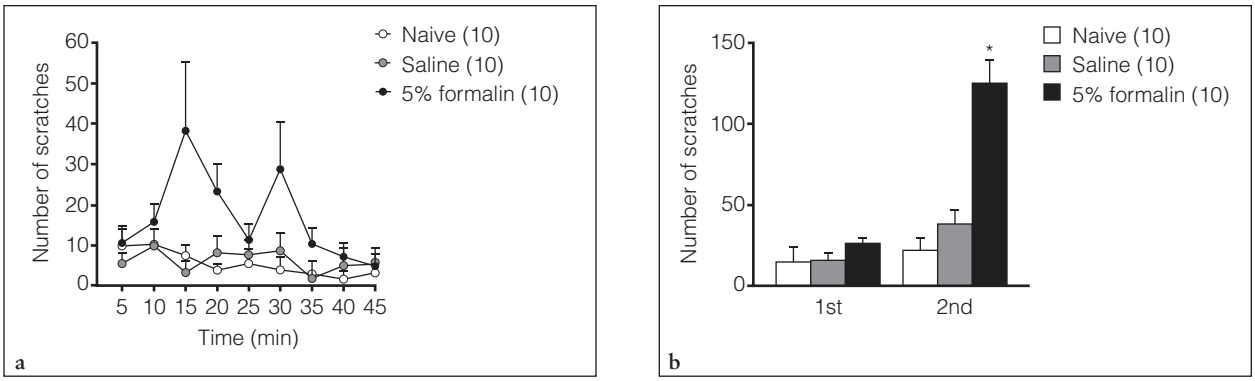
Rats (n = 4 per group) were sacrificed under isoflurane anesthesia 1 hour after formalin injection into the TMJ. The dorsal parts of the caudal medullae were then rapidly removed and frozen in liquid nitrogen. Samples were sonicated with Biorupture (Cosmo Bio) in lysis buffer containing a protease and phosphatase inhibitor cocktail (Thermo Scientific). For western blot analysis, total proteins (30 μg) were separated on a 4% to 12% gradient NuPAGE Novex Bis-Tris gel (Invitrogen) and transferred onto a polyvinylidene difluoride (PVDF) membrane using the iBlot Dry blotting system (Invitrogen). The membranes were blocked with 5% nonfat milk in tris-buffered saline (TBS) with 0.1% Tween 20 for 1 hour at room temperature and then incubated with rabbit anti-phospho-p38, p38 MAPK (1:1000; Cell Signaling Technology), or rabbit anti-Iba1 (ionized calcium binding adaptor molecule 1, activated microglial marker, 1:500; Wako Chemical) at 4°C overnight. The present study used Iba1 antibody rather than OX42 for the western blot analysis because Iba1 is a more sensitive and specific marker of the induction of the reactive profile in microglia,[15–18] as described previously.[17,19] GAPDH (glyceraldehyde 3-phosphate dehydrogenase, 1:5000; Santa Cruz Biotechnology) antibody was used as a loading control. The blots were then incubated with goat anti-rabbit horseradish peroxidase conjugated antibody for 2 hours at room temperature. Membranes were developed using the SuperSignal West Femto substrate (Pierce) and exposed to x-ray film. A computer image analysis system (ImageJ, NIH) was used for quantification of the specific bands.

Verification of Inflammation

To confirm that the plasma extravasation induced by the TMJ injections in the rats was indeed restricted to the TMJ region, formalin-induced plasma extravasation of the Evans' blue dye bound to the plasma protein was measured, as described previously.[11–13,20] At the conclusion of each experiment, the animals were anesthetized with pentobarbital sodium (40 mg/kg, ip). Evans' blue dye (0.1%, 5 mg/kg) was injected into the right femoral vein. Ten minutes after the injection of the dye, each rat was perfused with heparinized normal saline. Joint tissues were dissected from the left side, weighed, and stored at –20°C until analyzed. The tissues were incubated overnight in a 7:3 mixture of acetone and 5% sodium sulphate solution at room temperature with intermittent shaking. After incubation, the samples were centrifuged at 300 rpm for 10 minutes, and the supernatant was separated. The samples were analyzed for the amount of dye present by spectrophotometric measurement of the absorbance at 620 nm. The recovery of the extravasated dye per gram weight of tissue (μg/g) was calculated by comparing the absorbance of the supernatant with a standard curve. The standard curve was generated from a series of the same extraction solution mixed with known amounts of Evans' blue dye.

Rotarod Test

Changes in motor performance after the intracisternal administration of 50 μg of minocycline or 5 μg of SB203580 were measured using a rotarod (Ugo Basile, Comerio), as described previously.[12,21] The rotarod speed increased to 16 rpm over a 180-second period, with the maximum time spent on the rod set at 300 seconds. Rats received two or three training trials, on 2 separate days prior to testing, for acclimatization. On the experimental day, the time course of motor performance was



Figs 1a and 1b *(a)* Time course of the formalin-induced behavioral responses. The number of responses was measured for nine successive 5-minute intervals. *(b)* Formalin-induced scratching behavioral responses exhibited two phases, with an early short-lasting response (first phase: 0 to 10 minutes) and a continuous prolonged response (second phase: 10 to 45 minutes). There were 10 animals in each group. **P* < .05, saline- versus formalin-treated group.

examined before and after intracisternal administration of the inhibitors.

SB203580 and Minocycline Preparation and Use

SB203580 and minocycline, purchased from Sigma and Calbiochem, respectively, were dissolved in 20% dimethyl sulfoxide (DMSO) and 80% sterile saline. In the control group, the vehicle (20% DMSO and 80% sterile saline) for SB203580 or minocycline was injected intracisternally for comparison of drug modulation versus control responses.

Statistical Analysis

Statistical analysis of the behavioral and western blot data was carried out using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc analysis. In all statistical comparisons, $P < .05$ was considered to be statistically significant. All data are presented as the mean ± SEM.

Results

The present study demonstrated that intra-articular injection of 50 µL of 5% formalin significantly produced scratching responses indicative of nociceptive behavior (Fig 1). Intra-articular injection of the vehicle (saline) did not produce significant nociceptive behavioral responses as compared to the naive rats. In contrast, formalin injected intra-articularly significantly increased the total number of scratches from 38 ± 9 to 124 ± 15 scratches ($P < .05$) as compared to the vehicle-treated rats (Fig 1). TMJ inflammation was also confirmed by measuring the amount of Evans' blue dye in the TMJ, following intra-articular injection of formalin. The concentration of Evans' blue dye was significantly higher in the formalin-treated group (15.6 ± 2.8 µg, $P < 0.05$) as compared to the vehicle (saline)-treated group (3.6 ± 1.0 µg). Intra-articular injection of formalin did not increase the level of dye in the contralateral TMJ. Moreover, an intramuscular injection of formalin did not increase the amount of Evans' blue dye in the TMJ.

Figure 2 illustrates the expression of p-p38 MAPK immunoreactivity on the ipsilateral medullary dorsal horn 1 hour after formalin injection. Intra-articular injections of formalin increased the expression of p-p38 MAPK on the ipsilateral side of the medullary dorsal horn compared to the naive rat. However, differences in p-p38 MAPK expression were not observed on the contralateral side between the two groups. In addition, the p-p38 MAPK expression co-localized with OX42 (a microglial marker) and NeuN (a neuronal marker), but not with GFAP (an astrocyte marker). No immunostaining was observed in contol sections that were incubated without primary antibody (data not shown).

Figure 3 illustrates the effect of intracisternally-administered SB203580, a p38 MAPK inhibitor, on the number of scratches and p-p38 MAPK expression produced by formalin injection into the TMJ region. Intracisternal administration of the vehicle did not alter the formalin-induced scratching behavior. However, intracisternal injections of 0.5, 1, or 5 µg of SB203580 significantly attenuated the number of scratches by 49% (67 ± 5 scratches, $P < .05$), 53% (61 ± 11 scratches, $P < .05$), or 71% (38 ± 7 scratches, $P < .05$), respectively, compared to the vehicle-treated group (Fig 3a). Changes

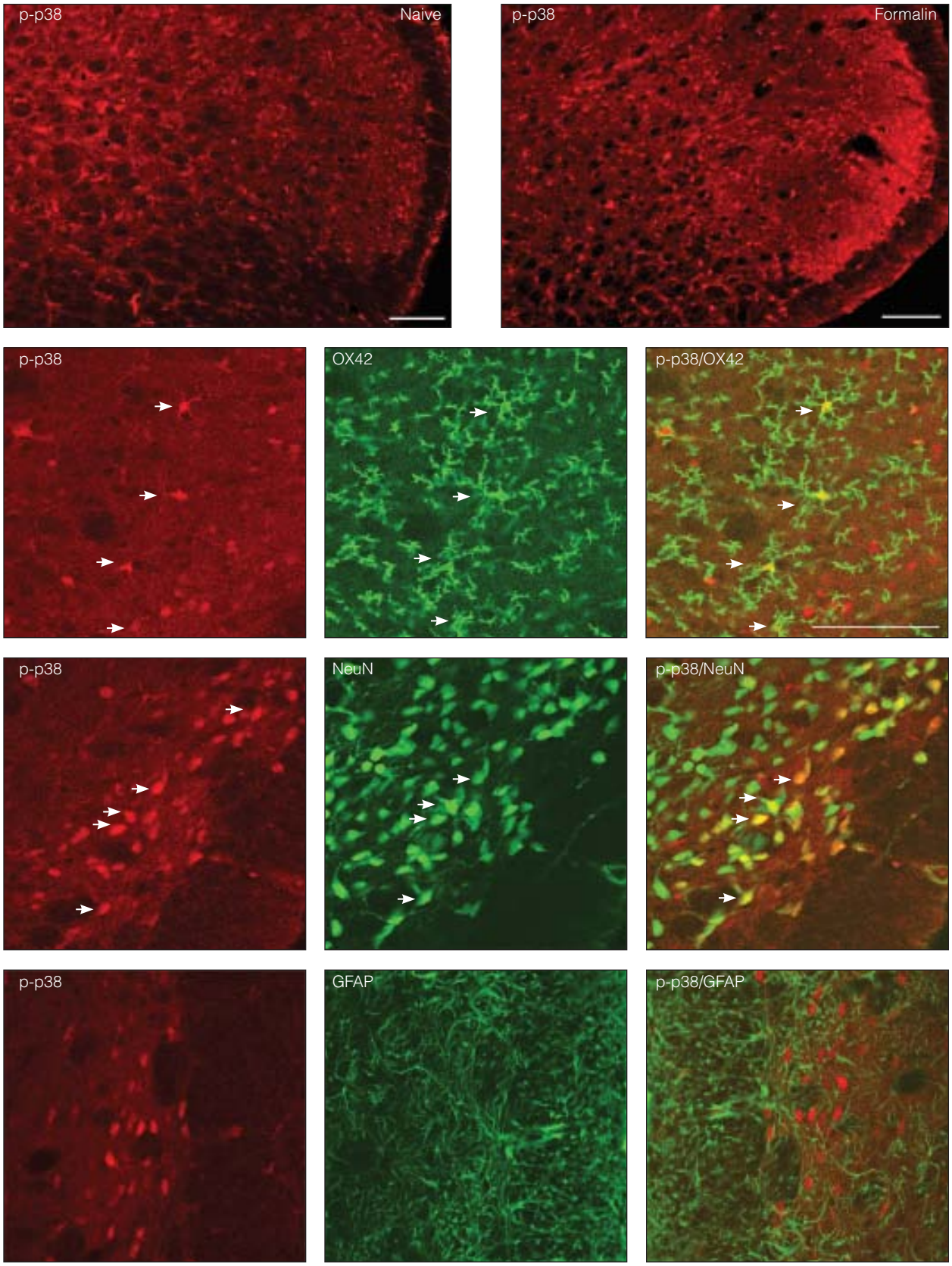


Fig 2 Expression of p-p38 MAPK immunoreactivity in the ipsilateral medullary dorsal horn following intra-articular injection of formalin into the TMJ. Double immunofluorescence is shown for p-p38 MAPK *(red)* and OX42, a microglia marker *(green)*, NeuN, a neuronal maker *(green)*, or GFAP, an astrocyte marker *(green)*. The confocal immunofluorescence images showed co-localization of p-p38 MAPK with OX42 and NeuN in the medullary dorsal horn. Scale bars, 100 μm.

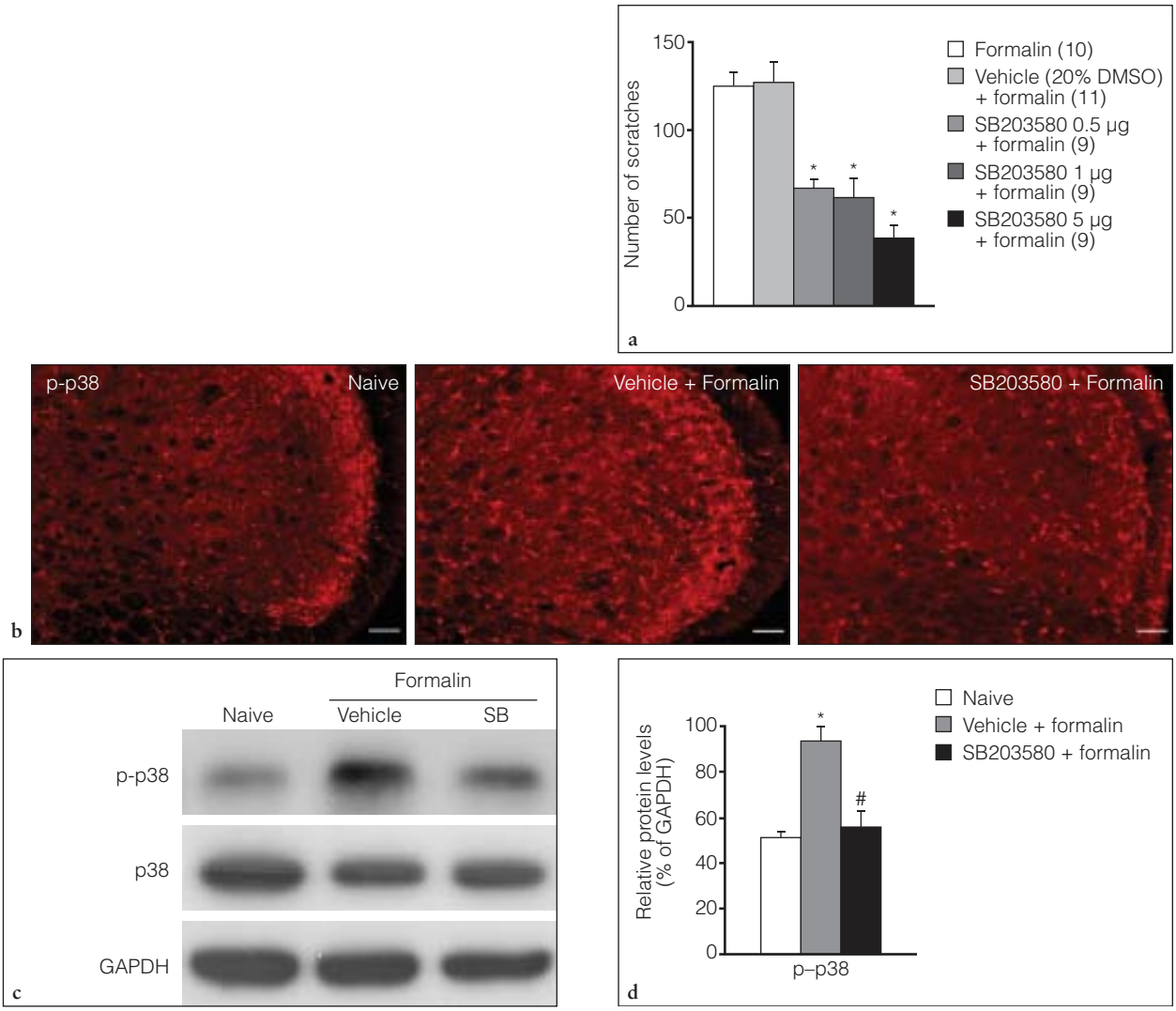


Fig 3 Effects of treatment with SB203580, a p38 MAPK inhibitor, on formalin-induced TMJ nociception. *(a)* Intracisternal administration of SB203580 attenuated the formalin-induced scratching behavior compared to the vehicle-treated group. **P* < .05, vehicle- versus SB203580-treated group. *(b)* Intracisternal administration of SB203580 (5 µg) suppressed the expression of p-p38 MAPK produced by formalin injection. *(c and d)* Western blot analysis confirmed that the increased p-p38 MAPK levels were significantly reversed following administration of SB203580 (SB), compared to the vehicle treatment. However, levels of p38 MAPK did not change significantly, and levels of GAPDH (used as loading control) were constant in all groups. **P* < .05, naive- versus vehicle + formalin-injected group. #*P* < .05, vehicle + formalin- versus SB203580 + formalin-treated group. Scale bars, 100 µm.

in the p-p38 MAPK expression in the medullary dorsal horn following the administration of SB203580 (5 µg) to the rats are shown in Fig 3b. Intra-articular injection of formalin increased p-p38 MAPK expression on the ipsilateral side. Intracisternal administration of SB203580 attenuated the expression of p-p38 MAPK compared to the vehicle-treated rats (Fig 3b). Western blot analysis further demonstrated that the increase in p-p38 MAPK levels was significantly reversed following the administration of SB203580 compared to the vehicle treatment (Figs 3c and 3d).

Figure 4 illustrates the effects of minocycline, a microglia inhibitor, on the nociceptive behavior, microglia activation, and p-p38 MAPK expression produced by formalin injection. Intracisternal administration of the vehicle did not alter the formalin-induced scratching behavior. However, intracisternal injections of 25 or 50 µg of minocycline attenuated the number of scratches by 43% (70 ± 13 scratches, *P* < .05) or 60% (50 ± 13 scratches, *P* < .05), respectively, compared to the vehicle-treated group (Fig 4a). Intracisternal administration of minocycline (50 µg) attenuated the expression

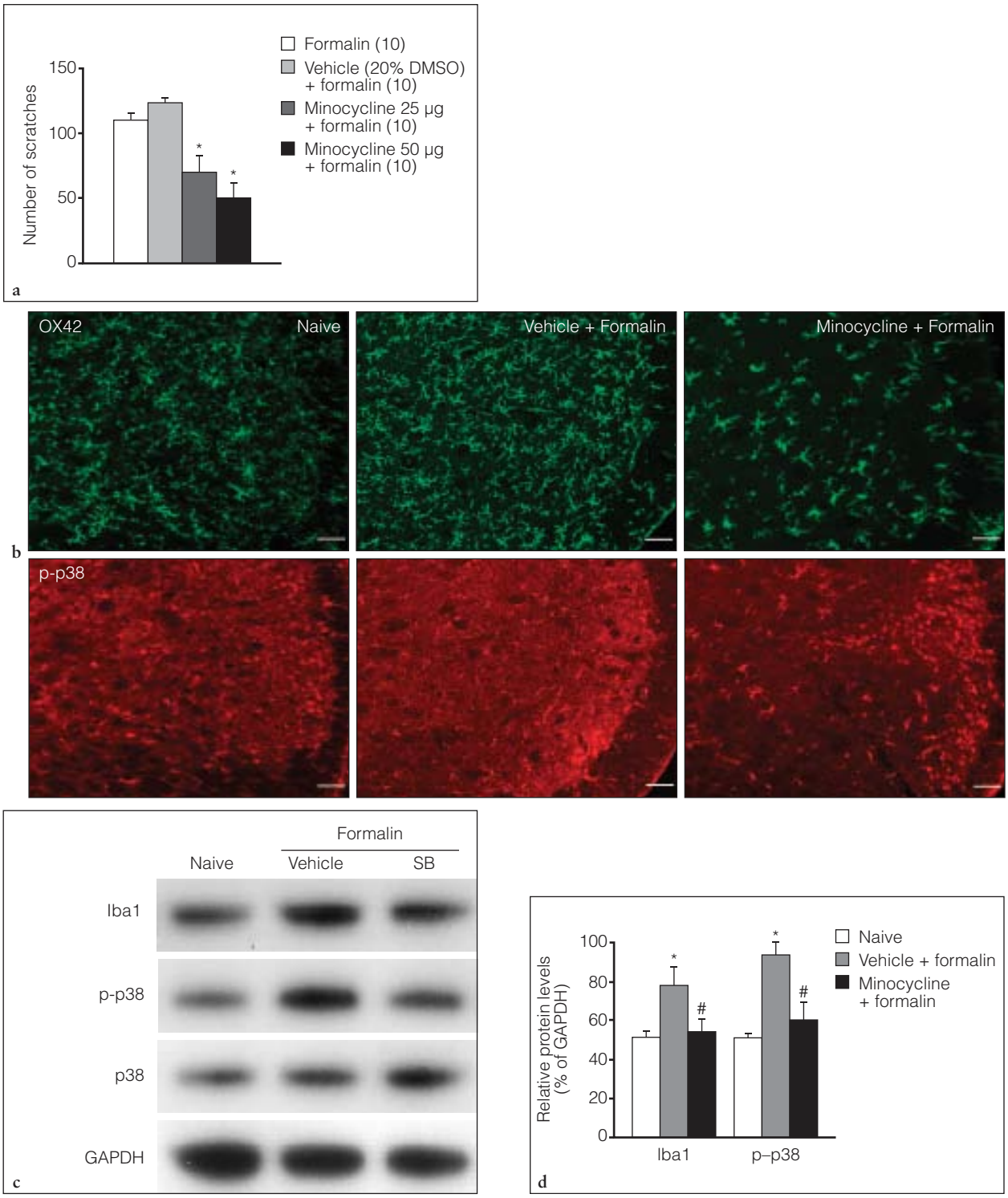


Fig 4 Effects of treatment with minocycline, a microglia inhibitor, on formalin-induced TMJ nociception. *(a)* Intracisternal administration of minocycline attenuated the formalin-induced scratching behavior, compared to the vehicle-treated group. **P* < .05, vehicle- versus minocycline-treated group. *(b)* Intra-articular injections of formalin increased OX42 (upper) or p-p38 MAPK (lower) immunoreactivity on the ipsilateral medullary dorsal horn, compared to the naive rat. Intracisternal administration of SB203580 (5 µg) suppressed the OX42 or p-p38 MAPK immunoreactivity. *(c and d)* Western blot analysis confirmed that the increased OX42 or p-p38 MAPK levels were significantly reversed following administration of minocycline (mino) compared to the vehicle control treatment. However, levels of p38 MAPK did not change significantly, and levels of GAPDH (used as loading control) were constant in all groups. **P* < .05, naive versus vehicle + formalin-injected group. #*P* < .05, vehicle + formalin- versus SB203580 + formalin-treated group. Scale bars, 100 µm.

of OX42, a marker of microglia, produced by the intra-articular injection of formalin. Moreover, minocycline injected intracisternally attenuated the expression of p-p38 MAPK in the medullary dorsal horn (Fig 4b). Western blot analysis further demonstrated that the increased levels of Iba1 (a marker of microglia) and p-p38 MAPK were significantly reversed following administration of minocycline (50 μg) compared to the vehicle treatment (Figs 4c and 4d). Changes in motor performance after intracisternal administration of SB203580 or minocycline were measured using the rotarod test. The highest dose of SB203580 (5 μg) or minocycline (50 μg) intracisternally injected did not affect the rotarod function (data not shown).

Discussion

These findings demonstrate that intra-articular injections of formalin into the TMJ activates p38 MAPK in microglia of the medullary dorsal horn and that this is accompanied by nociceptive behavior. In addition, intracisternal administration of minocycline attenuates nociceptive behavior and inhibits microglial p38 MAPK activation. These results indicate that microglial p38 MAPK participates in the central processing of inflammatory TMJ nociception. Thus, a targeted blockade of the microglial p38 MAPK pathway could be a potentially important new treatment strategy for inflammatory TMJ nociception.

Previously, animal models for TMJ pain were developed by the injection of complete Freund's adjuvant,[22,23] mustard oil,[24–26] glutamate,[25,26] or substance P[27] into the TMJ region of rats. Although these experimental models allowed for the study of mechanisms underlying these inflammatory and pain conditions, they involved only evaluation of the muscle activity or nociceptive behavior evoked from the skin overlying the TMJ inflammation. Recently, however, a formalin-induced TMJ pain model in rats was described in which microinjection of formalin into the TMJ region produces nociceptive scratching behavior.[11–13,28]

Microglia represent the resident immune cells of the central nervous system, which are activated by neurotransmitters that are released from the central terminals of activated peripheral nerves and modulate both neuronal and astrocyte function. There is accumulating evidence that glial cells in the central nervous system, activated by inflammation or peripheral nerve injury, are involved in spinal nociceptive transmission and central sensitization. Inflammatory pain has been found to increase microglial activity in the spinal cord,[29] and the intrathecal administration of minocycline, a microglia inhibitor, attenuated thermal hyperalgesia and/or allodynia as a result of decreased microglial activation.[30] Peripheral inflammation or nerve injury also increases the number of p-p38 MAPK-labeled microglia,[31] and intrathecal pretreatment with p38 MAPK inhibitors attenuates the hyperalgesia evident in rats with nerve injury or inflammation associated with central sensitization in the medullary[7] and spinal dorsal horn.[32,33] These results suggest that microglial p38 MAPK is involved in inflammation-induced pain and has an important role in nociceptive processing and sensitization. The present study has demonstrated that intracisternal injections of SB203580 significantly attenuated the number of scratches and the expression of p-p38 MAPK in the medullary dorsal horn produced by intra-articular injection of formalin into the TMJ. These results provide evidence that activation of p38 MAPK in the medullary dorsal horn has a role in formalin-induced TMJ nociception.

The study also demonstrated the localization of p-p38 MAPK in the microglia following formalin injection into the TMJ of rats. Moreover, intracisternal injections of minocycline attenuated the number of scratches and expression of OX42 and p-p38 MAPK in the medullary dorsal horn produced by the intra-articular injection of formalin. The present results suggest that microglial p38 MAPK has a role in formalin-induced TMJ nociception since blocking its activation by pretreatment with minocycline, a microglia inhibitor, effectively prevented the scratching behavior. Intracisternal administration of 50 μg of minocycline as well as 5 μg of SB203580 did not affect motor functions in the present investigation, suggesting that the antinociceptive effects produced by intracisternal administration of p38 MAPK or microglia inhibitor are not associated with motor impairment.

The relationship between p38 MAPK phosphorylation and microglial activation during nociceptive sensitization has already been well established. Most pain models, including those using formalin,[31,34] capsaicin injection,[32] nerve injury,[33,35] and spinal cord injury[36] have been shown to induce spinal p38 MAPK phosphorylation in microglia. These earlier results are consistent with the present findings that microglial p38 MAPK plays an important role in formalin-induced TMJ nociception. Previous studies have also demonstrated that glial metabolic processes are important in the development of central sensitization in the medullary dorsal horn.[5,7,37,38] Intrathecal application of SB203580, an inhibitor of p38 MAPK, and fluoroacetate, an inhibitor of the

astroglial metabolic enzyme, markedly attenuated the mustard oil–induced increases in pinch mechanoreceptive field size and the responses of nociceptive neurons in the medullary dorsal horn to the application of mustard oil to the tooth pulp, whereas administration of either drug did not significantly affect the baseline mechanoreceptive field size and response properties.[7] Recently, immune cells have been shown to not only contribute to immune protection but also to be involved in the sensitization of peripheral nociceptors. The immune cells along with glia and neurons modulate the excitability of the nociceptive pathways through the synthesis and release of inflammatory mediators and through interactions with neurotransmitters and their receptors.[39] It has been well documented that glia dynamically regulate neuronal synaptic communications and can produce nociceptive behavior by the release of a variety of neurotransmitters, neuromodulators, and proinflammatory cytokines and chemokines.[40] Astrocytes are also involved in the enhanced nociceptive responses of the medullary dorsal horn nociceptive neurons and in the associated orofacial hyperalgesia following trigeminal nerve injury or inflammation.[8,37] Therefore, the role of glia in central sensitization and its association with the action of immune cells requires future study.

The present data further demonstrated that p-p38 MAPK expression co-localizes with NeuN, a neuronal marker. TMJ formalin injection experiments revealed the localization of p-p38 MAPK in neurons, in agreement with previous observations in adjuvant immunized rats,[41] venom-induced inflammation,[42] an incisional pain model,[43] and a neuropathic pain model.[44] Previous studies demonstrated that p38 MAPK mediates sensitization of primary and second-order nociceptive neurons by increasing membrane receptor expression, ion channel expression and activity, and stimulating synthesis and secretion of several mediators that promote or maintain neuronal hyperexcitability.[45–47] Moreover, spinal administration of p38 MAPK inhibitor can suppress not only the production of several cytokines, including IL-1β, tumor necrosis factor-α (TNF-α), and IL-6 but also spinal nerve injury-induced allodynia.[31,33,35] These results collectively suggest that neuronal p38 MAPK also participates in the central processing of inflammatory TMJ nociception.

In the present study, the intracisternal administration of minocycline, a microglia inhibitor, attenuated nociceptive behavior as well as the activation of microglia following formalin injection into the TMJ. Moreover, minocycline was found to inhibit the formalin-induced expression of p-p38 MAPK in the medullary dorsal horn. These results suggest that formalin-induced TMJ inflammation produces nociceptive behavior through the activation of p38 MAPK in microglia of the medullary dorsal horn and that p38 MAPK has an important role in glia-mediated pain transmission. Moreover, these results suggest that microglia, which are now recognized as powerful modulators of pain, will continue to emerge as a putative new target for drug development.

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