Biochemical and Biophysical Research Communications 421 (2012) 318–322

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The critical role of spinal ceramide in the development of partial sciatic nerve ligation-induced neuropathic pain in mice

Yuka Kobayashi [a], Norikazu Kiguchi [a], Takehiko Maeda [b], Masanobu Ozaki [c], Shiroh Kishioka [a,*]

[a] Department of Pharmacology, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-0012, Japan
[b] Department of Pharmacology, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Niigata 956-8603, Japan
[c] Department of Toxicology, Niigata University of Pharmacy and Applied Life Sciences, 265-1 Higashijima, Niigata 956-8603, Japan

ARTICLE INFO

Article history:
Received 29 March 2012
Available online 7 April 2012

Keywords:
Sphingolipids
Allodynia
Microglia
Cytokines
Dorsal horn

ABSTRACT

Recent observations indicate that peripheral nerve injury induces central sensitization through microglial activation and the release of inflammatory cytokines, resulting in the development of neuropathic pain. However, the underlying mechanisms of this phenomenon remain to be fully elucidated. In this study, we examined the involvement of spinal ceramide, a bioactive lipid, in the development of neuropathic pain induced by partial sciatic nerve ligation (PSL). We found that the mRNA expression levels for ceramide synthase and neutral sphingomyelinase, which are enzymes of ceramide biosynthesis, were up-regulated in the spinal cord from 3 h to 1 day after PSL. The mRNA expressions of cytokines (interleukin-1β and tumor necrosis factor-α) and the microglial specific molecules (Iba-1 and CD11b) were also increased in the spinal cord after PSL. In the von Frey test, intrathecal injection of the ceramide biosynthesis inhibitors Fumonisin B1 and GW4869 at 3 h and day 3 after PSL significantly attenuated PSL-induced tactile allodynia. By immunohistochemistry, microglial activation in the dorsal horn was suppressed by Fumonisin B1 and GW4869. Therefore, we conclude that spinal ceramide may play a crucial role in PSL-induced neuropathic pain through the activation of microglia.

1. Introduction

Neuropathic pain occurs after injuries to the central or peripheral nervous systems and is characterized by spontaneous pain, excessive responses to painful stimuli (hyperalgesia), and pain responses to innocuous stimuli (allodynia) [1]. The molecular mechanisms of neuropathic pain are poorly understood, and treating neuropathic pain with several standard analgesics has limited success [2]. Therefore, there is a strong clinical need to develop novel and effective treatments for neuropathic pain.

Previous research using several animal models of neuropathic pain has indicated that the relationship between inflammatory and immune mechanisms in the central and the peripheral nervous systems plays important roles in neuropathic pain [3,4]. Recently, a number of studies have focused on glial cells in the spinal cord because it is believed that they may contribute to the pathogenesis of neuropathic pain. Activated spinal microglia and astrocytes release various inflammatory mediators, such as cytokines, chemokines, and growth factors. These mediators facilitate pain transmission and contribute to the development of neuropathic pain. [4–6].

It has been reported that several bioactive lipid mediators – including eicosanoide, diacylglycerol, lysophospholipid, and sphingolipid – enhance the pain response [7–11]. Ceramide is a member of the sphingolipid family and is the central hub of sphingolipid metabolism. It is synthesized *de novo* by ceramide synthase (CERS) or generated from the hydrolysis of sphingomyelin by both acid and neutral sphingomyelinase (SMase).

Intradermal injection of ceramide into the rat hind paw produces hyperalgesia [12]. Moreover, chronic morphine-induced antinociceptive tolerance is attenuated by ceramide biosynthesis inhibitors [13]. These results indicate that ceramide is closely related to pain regulation. Previous studies have also provided evidence that ceramide activates primary microglial cells [14]. Although, as described above, ceramide is involved in pain regulation and the activation of glial cells, it is unclear how ceramide contributes to the development of neuropathic pain. In the present study, we investigated the involvement of ceramide in neuropathic pain induced by partial sciatic nerve ligation (PSL).

Abbreviations: CERS, ceramide synthase; DMSO, dimethyl sulfoxide; FB1, Fumonisin B1; IL-1β, interleukin-1β; i.t., intrathecal; PBS, phosphate buffered saline; PSL, partial sciatic nerve ligation; SCN, sciatic nerve; SMase, sphingomyelinase; TNF-α, tumor necrosis factor-α.

* Corresponding author. Fax: +81 73 446 3806.
E-mail addresses: koba@wakayama-med.ac.jp (Y. Kobayashi), kiguchi@wakayama-med.ac.jp (N. Kiguchi), maeda@nupals.ac.jp (T. Maeda), ozaki@nupals.ac.jp (M. Ozaki), kishioka@wakayama-med.ac.jp (S. Kishioka).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.03.153

2. Materials and methods

2.1. Animals

Male ICR mice (4 or 5 weeks old; 18–25 g body weight) were used for all experiments (SLC, Osaka, Japan). Mice were housed under controlled ambient (23–24 °C, 60–70% relative humidity) and light (light on from 8:00 AM to 8:00 PM) conditions in our institutional vivarium, and they had *ad libitum* access to water and food. All experimental procedures were approved by the Animal Research Committee of Wakayama Medical University (approval No. 395) and complied with the Ethical Guidelines of the International Association for the Study of Pain [15].

2.2. Chemicals and administration technique

The drugs we used were Fumonisin B1 (inhibitor of ceramide synthase; Tocris Bioscience, Ellisville, MO), GW4869 (inhibitor of neutral SMase; Sigma–Aldrich, Tokyo, Japan). These drugs were referred to previous report [13,16,17]. For intrathecal (i.t.; 5 μl) injections, Fumonisin B1 and GW4869 were dissolved in physiological saline containing 20% dimethyl sulfoxide (DMSO). Each i.t. injection was performed free hand between spinal L5 and L6 segments, according to previous report [18].

2.3. Partial sciatic nerve ligation

PSL was performed as previously described [19]. Briefly, under intraperitoneal sodium pentobarbital (70 mg/kg, nacalai tesque, Kyoto, Japan) anesthesia, the common sciatic nerve was exposed through a small incision. The location of the sciatic nerve (SCN) ligation was identified using the femoral head as a landmark. Approximately 1/2 of the SCN thickness was tightly ligated with a silk suture. Exposure of the SCN was performed as a sham control operation. The incision was closed with a suture and disinfected by povidone-iodine.

2.4. von Frey test

Tactile allodynia was evaluated using von Frey filaments (Neuroscience, Tokyo, Japan.). Mice were placed on a 5 × 5 mm wire mesh grid floor and covered with an opaque cup to avoid visual stimulation. Before the test, the mice were allowed to adapt for 2–3 h. The von Frey filament was applied to the middle of the planter surface of the hind paw with a weight of 0.16 g. On the indicated days, withdrawal responses following stimulation of the hind paw were measured 10 times. Tactile allodynia was defined as an increase in the number of withdrawal responses to the stimulation.

2.5. RT-PCR

For the RT-PCR assays, we collected lumbar spinal cords. Total RNA was purified using the isolated spinal cord and was converted to cDNA by reverse transcription. Ten nanograms of cDNA and the following primers were used for PCR: neutral SMase 2 (5′-ACC TCA ATA TGC ACC CCA AA-3′ and 5′-TTA GCA CGC TGA TCA AAT CG-3′), CERS (5′-ACC TGG GCT GAT CTA GAA GAC A-3′ and 5′-TCT GCC GTG ACA AAA GGT CT-3′), interleukin-1β (IL-1β; 5′-CAG GCA GGC AGT ATC ACT CA-3′ and 5′-CAG GCA GGC AGT ATC ACT CA-3′), tumor necrosis factor-α (TNF-α; 5′-GAG CAC AGA AAG CAT GAT CC-3′ and 5′-CAC TTG GTG GTT TGC TAC GA-3′), Iba-1 (5′-GGA TTT GCA GGG AGG AAA AG-3′ and 5′-AGA TCT CTT GCC CAG CAT CA-3′), CD11b (5′-GTG CTG AGA CTG GAG GCA AC-3′ and 5′-TCC ACG CAG TCC GGT AAA AT-3′), GAPDH (5′-TCC ATG ACA ACT TTG GCA TTG TGG-3′ and 5′-GTT GCT GTT GAA GTC GCA GGA GAC-3′). All primers were purchased from Operon Biotechnology (Tokyo, Japan). Electrophoresis of the PCR products on 1.5% agarose gel was visualized by ethidium bromide staining and photographed. The fluorescent intensities of gray scale image were analyzed using Image J software (National Institutes of Health, Bethesda, MD) and normalized by GAPDH.

2.6. Immunohistochemistry

Mice were deeply anesthetized with intraperitoneal sodium pentobarbital (100 mg/kg) and perfused transcardially with 30 ml of phosphate buffered saline (PBS), followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The collected L5–L6 spinal cords were postfixed in 4% paraformaldehyde and then dehydrated overnight in 25% sucrose at 4 °C. The tissue samples were frozen in optimal cutting temperature compound (SAKURA Finetechnical Co., Tokyo, Japan) and sliced longitudinally at 15 μm, using a cryostat (Leica Microsystems., Wetzlar, Germany). The sections were incubated with 4% bovine serum albumin in PBS containing 0.1% Triton X-100 (PBST) at room temperature for 2 h, and incubated with a specific antibody against Iba-1 (specific antibody for microglia; rabbit polyclonal IgG, 1:250, Wako, Osaka, Japan) [20] at 4 °C overnight. The next day, sections were incubated for 2 h in a specific secondary antibody conjugated with fluorescent markers (Alexa-488-conjugated donkey anti-rabbit IgG, Invitrogen, Carlsbad, CA), and followed by nuclear staining using Hoechst 33342 (Invitrogen). Finally, a coverslip with Prema Flour (Thermo Fisher Scientific, Pittsburgh, PA) was placed over each section. Immunoreactivity was detected with a fluorescence microscope (Nikon, Tokyo, Japan). The Iba-1 positive cells which are overlapped with nuclei and exceed fixed intensity (threshold:140) and size (particle:0–100) in a square of 300 × 300 μm in gray scale image were automatically counted using Image J software.

2.7. Statistical analysis

Data are presented as the mean ± S.E.M. and statistically analyzed using one-way analysis of variance, with post hoc correction for multiple comparisons using a Tukey's test or a Student's *t*-test. Statistical significance was arbitrated at $P < 0.05$.

3. Results

3.1. Increases in mRNA expressions of ceramide biosynthesis enzymes, cytokines, and microglial specific molecules by PSL

To determine the activation of ceramide biosynthesis in the spinal cord after PSL, we evaluated the mRNA expression of ceramide biosynthesis enzymes using RT-PCR. The mRNA expressions of CERS and neutral SMase2 were up-regulated at 3 h and 1–3 days after PSL (Fig. 1A) and at 3 h and 1 day after PSL (Fig. 1 B), respectively. Moreover, the mRNA expressions of the inflammatory cytokines (IL-1β and TNF-α) (Fig. 2A and B) and the microglial specific molecules (Iba-1 and CD11b) (Fig. 2C and D) were up-regulated in the spinal cord after PSL as compared with control (naive).

3.2. Attenuation of PSL-induced tactile allodynia by ceramide biosynthesis inhibitors

To investigate the involvement of spinal ceramide in pain transmission, we examined the effect of i.t. injection of CERS inhibitor Fumonisin B1 (FB1) on PSL-induced tactile allodynia. PSL elicited a significant increase in withdrawal responses to von Frey filaments, indicating the development of tactile allodynia. A single

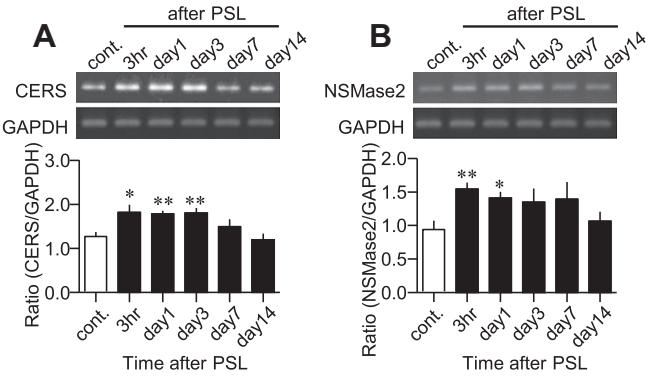

Fig. 1. The mRNA expression of ceramide biosynthesis enzymes in the spinal cord after partial sciatic nerve ligation (PSL). Mice received a PSL operation and their spinal cords were collected at 3 h, 1 day, 3 days, 7 days, or 14 days after PSL. The time courses of ceramide synthase (CERS) or neutral sphingomyelinase 2 (NSMase 2) mRNA expressions were evaluated by RT-PCR (CERS (A), NSMase 2 (B)). The level of gene expression in each column was normalized to GAPDH expression by Image J and is presented as relative mRNA expression units. Data are shown as the mean ± S.E.M. of 6–10 mice. vs. control (naive); $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

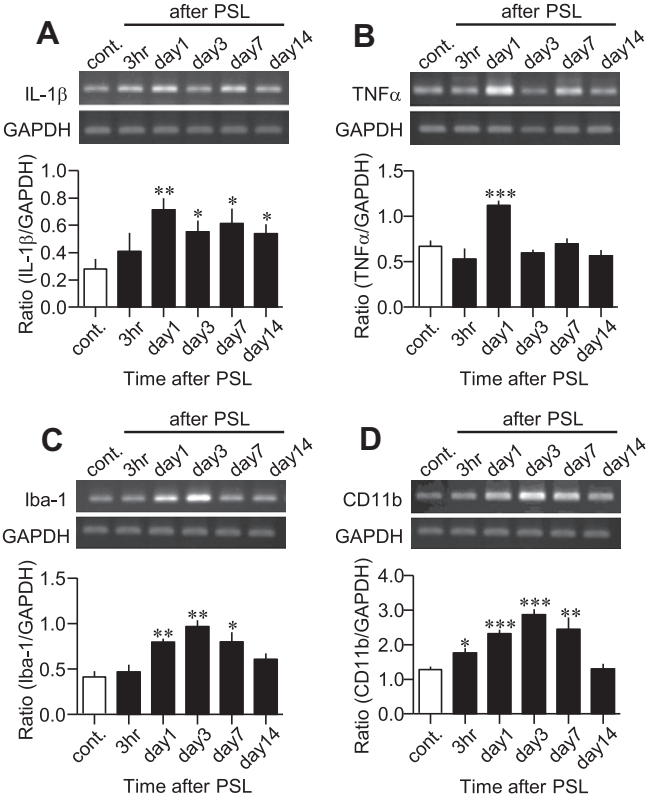

Fig. 2. The mRNA expression of inflammatory molecules in the spinal cord after partial sciatic nerve ligation (PSL). The upper graphs show the inflammatory cytokines (interleukin-1β; IL-1β (A) and tumor neurosis factor-α; TNF-α (B)) and the lower graphs shows the specific microglial molecules (Iba-1 (C) and CD11b (D)). Mice received a PSL operation and their spinal cords were collected at 3 h, 1 day, 3 days, 7 days, or 14 days after PSL. The level of gene expression in each column was normalized to GAPDH expression by Image J and is presented as relative mRNA expression units. Data are shown as the mean ± S.E.M. of 6–10 mice. vs. control (naive); $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

i.t. injection of FB1 (10 nmol/5 μl) at 3 h after PSL transiently attenuated PSL-induced tactile allodynia on day 2 (Fig. 3A), whereas a single i.t. injection of FB1 on day 7 after PSL had no significant effect (Fig. 3B). Furthermore, by twice i.t. injections (at 3 h and day 3 after PSL) of FB1, tactile allodynia was significantly attenuated on days 2–7 after PSL (Fig. 3C). On day 4 after PSL, the twice i.t. injections of FB1 dose-dependently suppressed PSL-induced tactile allodynia (Fig. 3D). Similarly, on day 4 after PSL, twice i.t. injections (at 3 h and day 3 after PSL) of the neutral SMase inhibitor GW4869 significantly attenuated PSL-induced tactile allodynia (Fig. 3E).

3.3. Suppression of spinal microglial activation after PSL by treatment with ceramide biosynthesis inhibitors

We examined the effects of FB1 and GW4869 on the activation of spinal microglia in the dorsal horn after PSL (Fig. 4). FB1 and GW4869 were i.t. injected two times at 3 h and day 3 after PSL, and the spinal cord was collected on day 4 after PSL. By immunohistochemistry, PSL-induced increase in Iba-1 positive cells in the spinal dorsal horn was significantly attenuated by the i.t. injection of FB1 (Fig. 4A and C). Similarly, i.t. injection of GW4869 suppressed the expression of Iba-1 positive cells (Fig. 4B and D). These inhibitors had no effect on the expression of Iba-1 positive cells in the dorsal horn of sham-operated mice.

4. Discussion

In this study, we showed that spinal ceramide plays a crucial role in the development of PSL-induced neuropathic pain through activating spinal microglia. This assertion is supported by the following results; (1) PSL induced increases in the mRNA expression of ceramide biosynthesis enzymes in the spinal cord, and (2) the tactile allodynia and spinal microglial activation after PSL were attenuated by i.t. injection of ceramide biosynthesis inhibitors.

Ceramide is produced by the synthetic enzymes, CRES and SMase. Among four isoforms of SMase, acid SMase is initially identified in mammal [21], and it is well-known that the deficiency of this enzyme causes Niemann-Pick disease [22,23]. Neutral SMase is subdivided into three types, which are neutral SMase1, 2, and 3. The functions of neutral SMase1 and neutral SMase3 are not entirely elucidated. On the other hand, neutral SMase2 is mostly studied and has been considered as the key enzyme in ceramide production. Neutral SMase2 is highly expressed in central nervous systems and implicated in a wide range of cell responses such as synaptic plasticity and cytokine-induced inflammation [24,25]. Therefore, we hypothesize that ceramide is increased in the spinal cord after PSL through the activation of CERS and neutral SMase2, which may contribute to the development of neuropathic pain. We found increases in the mRNA expression of ceramide biosynthesis enzymes (CERS and neutral SMase2) in the spinal cord at 3 h and 1 day after PSL. On behavioral analysis, single i.t. injection of ceramide biosynthesis inhibitors (FB1 or GW4869) in the early phase transiently attenuated tactile allodynia, whereas injection in the late phase did not. In addition, twice i.t. injection in the early phase (3 h and day 3 after PSL) showed effective suppression of tactile allodynia in comparison with single i.t. injection. These results may suggest that PSL elicited neuropathic pain through the increment of spinal ceramide in the early phase.

In neuropathic pain state, several inflammatory cytokines are increasing in the spinal cord as well as in the injured peripheral nerves [4,26]. In this study, the mRNA expressions of the inflammatory mediators (IL-1β and TNF-α) and the microglial specific molecules (Iba-1 and CD11b) were up-regulated in the spinal cord after PSL. These molecules have been reported to contribute to hypersensitivity after peripheral nerve injury and microglial activation [27–29]. The activation of spinal microglia is regarded to be a key factor in the development of neuropathic pain. Spinal microglial activation after peripheral nerve injury produces and releases various inflammatory mediators, which engender severe pain [29,30]. Our result were consistent with previous studies

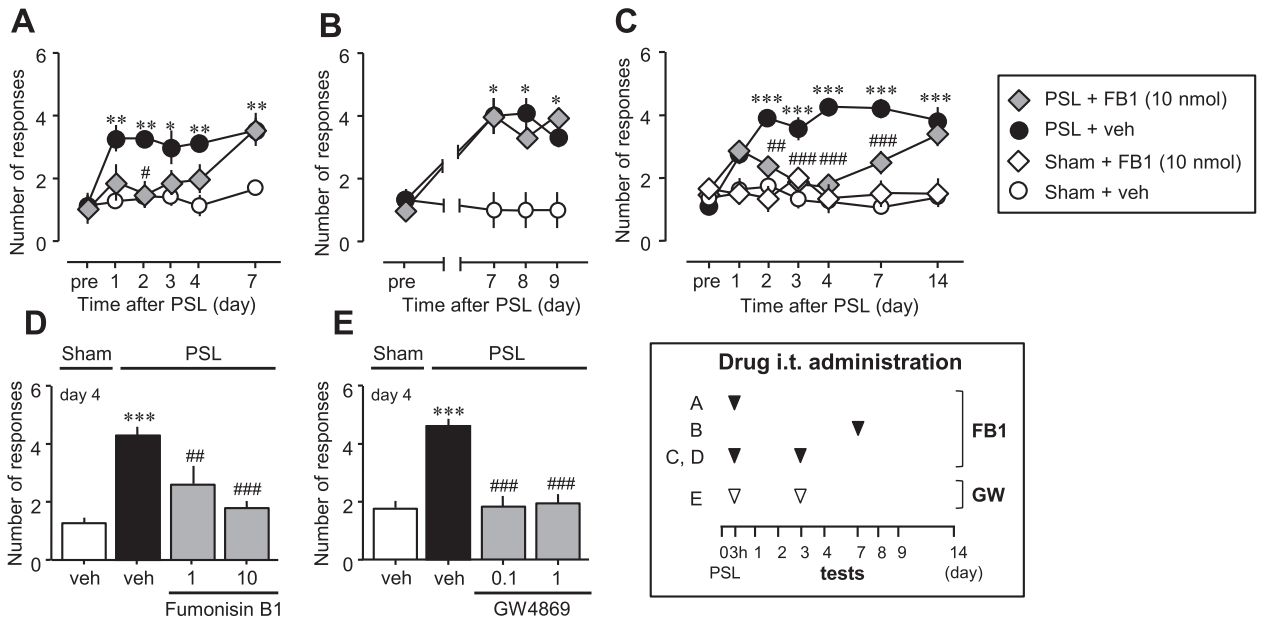

Fig. 3. Attenuation of partial sciatic nerve ligation (PSL)-induced tactile allodynia by treatment with inhibitors of ceramide biosynthesis. Tactile allodynia was evaluated by von Frey test. Mice received PSL or sham operations. Vehicle (veh; 20% DMSO), Fumonisin B1 (FB1; 1–10 nmol/5 μl, i.t.) or GW4869 (GW; 0.1–1 nmol/5 μl, i.t.) was injected according to time schedules shown right bottom. Time course (A, B and C) and dose-dependence (D, E) of suppressive effects of FB1 or GW on PSL-induced tactile allodynia were examined. On days 3 (C) and 7 (B), tests were performed at 3 h after drug administration. Open circle; sham + veh, closed circle; PSL + veh, open diamond; sham + FB1, closed diamond; PSL + FB1. Data are presented as the mean ± S.E.M. of 5–17 mice. vs. sham; $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. vs. PSL + inhibitors (FB1 or GW); $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$.

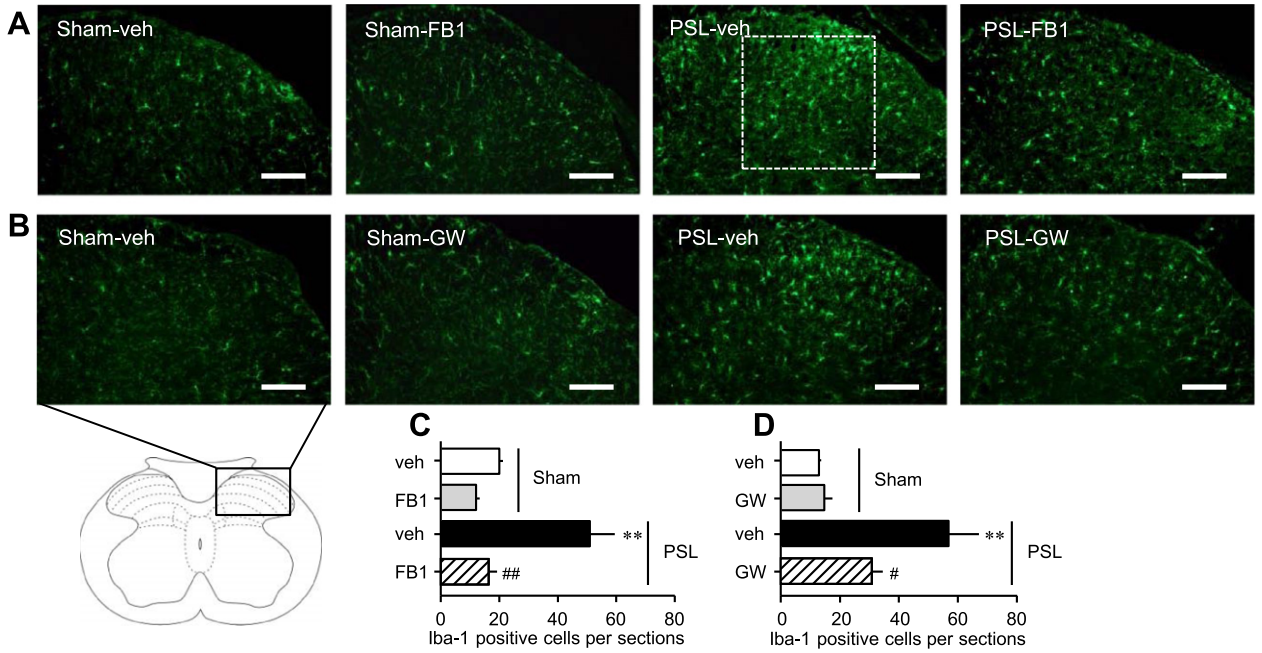

Fig. 4. Suppressive effects of Fumonisin B1 and GW4869 on partial sciatic nerve ligation (PSL)-induced microglial activation in the spinal dorsal horn. Mice received PSL or sham operations. Vehicle (veh; DMSO 20%), Fumonisin B1 (FB1; 10 nmol/5 μl) or GW 4869 (GW; 1 nmol/5 μl) was injected twice intrathecally at 3 h and day 3 after PSL. The expression of Iba-1 positive cells was visualized by immunohistochemistry in the spinal dorsal horn of operated side on day 4 after PSL or sham operation. Representative micrographs are presented (A: FB1 and B: GW). Quantitative analysis of Iba-1 positive cells was performed by Image J (C: FB1 and D: GW). Square in micrograph is 300 × 300 μm. Each column presents the mean ± S.E.M. of 3 mice. Scale bars = 100 μm. vs. sham + veh; $^{**}P < 0.01$. vs. PSL + inhibitors (FB1 or GW); $^{\#\#}P < 0.01$.

showing that ceramide activated cultured microglia and elicited the secretion of brain-derived neurotrophic factor and prostaglandin E_2 from microglia *in vitro* [14,31]. Both brain-derived neurotrophic factor and prostaglandin E_2 secretion from activated microglia are involved in central sensitization [32,33]. According to recent reports, ceramide is closely related to the production of these inflammatory mediators and plays an important role in peripheral sensitization through the induction of prostaglandin E_2 and TNF α [12,17]. Moreover, ceramide contributed to morphine-evoked hyperalgesia via the release of IL-1β, TNF α and IL-6 from activated glial cells [16]. These lines of evidence support a proposed mechanism that PSL increases endogenous ceramide through the activa-

tion of ceramide biosynthesis enzymes, and up-regulated ceramide activates microglia which releases inflammatory mediators in the spinal cord, leading to the development of neuropathic pain.

Previous reports have revealed that IL-1β, TNF-α, and nerve growth factor activate the hydrolysis of sphingomyelin and promote the generation of ceramide [34–36]. Thus, it is postulated that ceramide-induced inflammatory cytokines promote the hydrolysis of sphingomyelin, progressing ceramide generation. This process may be involved in the amplification mechanism of central sensitization and result in the development of neuropathic pain.

Although we need further studies to resolve the mechanisms of endogenous ceramide action in neuropathic pain, it has been reported that ceramide activates an intracellular signal transduction cascades that includes protein kinase C, protein phosphatase, and mitogen-activated protein kinase [37,38]. These cascades may be involved in ceramide-induced microglial activation, leading to neuropathic pain.

In conclusion, PSL accelerates ceramide biosynthesis in the spinal cord, and spinal ceramide activates microglia and releases inflammatory mediators, indicating the induction of central sensitization. These results suggest that ceramide may play a critical role in central sensitization, which is a critical pathogenic mechanism of neuropathic pain, and they open new avenues for the treatment of neuropathic pain.

Acknowledgments

This research was supported by a Grant-in-Aid for Research Activity start-up (20890200) from The Japan Society for the Promotion of Science.

References

[1] G. Moalem, D.J. Tracey, Immune and inflammatory mechanisms in neuropathic pain, Brain Res. Rev. 51 (2006) 240–264.
[2] S. Renfrey, C. Downton, J. Featherstone, The painful reality, Nat. Rev. Drug Discov. 2 (2003) 175–176.
[3] J. Scholz, C.J. Woolf, The neuropathic pain triad: neurons, immune cells and glia, Nat. Neurosci. 10 (2007) 1361–1368.
[4] F. Marchand, M. Perretti, S.B. McMahon, Role of the immune system in chronic pain, Nat. Rev. Neurosci. 6 (2005) 521–532.
[5] L.R. Watkins, S.F. Maier, Glia: a novel drug discovery target for clinical pain, Nat. Rev. Drug Discov. 2 (2003) 973–985.
[6] M. Tsuda, K. Inoue, M.W. Salter, Neuropathic pain and spinal microglia: a big problem from molecules in ''small'' glia, Trends Neurosci. 28 (2005) 101–107.
[7] J.L. Fuchs, J.A. Moore, H.D. Schwark, Peripheral inflammation increases phosphoinositide activity in the rat dorsal horn, Brain Res. 1003 (2004) 183–187.
[8] H. Vanegas, H.G. Schaible, Prostaglandins and cyclooxygenases [correction of cycloxygenases] in the spinal cord, Prog. Neurobiol. 64 (2001) 327–363.
[9] K.A. Park, M.R. Vasko, Lipid mediators of sensitivity in sensory neurons, Trends Pharmacol. Sci. 26 (2005) 571–577.
[10] M. Inoue, M.H. Rashid, R. Fujita, J.J. Contos, J. Chun, H. Ueda, Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling, Nat. Med. 10 (2004) 712–718.
[11] G.J. Patti, O. Yanes, L.P. Shriver, J.P. Courade, R. Tautenhahn, M. Manchester, G. Siuzdak, Metabolomics implicates altered sphingolipids in chronic pain of neuropathic origin, Nat. Chem. Biol. 8 (2012) 232–234.
[12] E.K. Joseph, J.D. Levine, Caspase signalling in neuropathic and inflammatory pain in the rat, Eur. J. Neurosci. 20 (2004) 2896–2902.
[13] M.M. Ndengele, S. Cuzzocrea, E. Masini, M.C. Vinci, E. Esposito, C. Muscoli, D.N. Petrusca, V. Mollace, E. Mazzon, D. Li, I. Petrache, G.M. Matuschak, D. Salvemini, Spinal ceramide modulates the development of morphine antinociceptive tolerance via peroxynitrite-mediated nitroxidative stress and neuroimmune activation, J. Pharmacol. Exp. Ther. 329 (2009) 64–75.
[14] K. Nakajima, Y. Tohyama, S. Kohsaka, T. Kurihara, Ceramide activates microglia to enhance the production/secretion of brain-derived neurotrophic factor (BDNF) without induction of deleterious factors in vitro, J. Neurochem. 80 (2002) 697–705.
[15] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.
[16] C. Muscoli, T. Doyle, C. Dagostino, L. Bryant, Z. Chen, L.R. Watkins, J. Ryerse, E. Bieberich, W. Neumman, D. Salvemini, Counter-regulation of opioid analgesia by glial-derived bioactive sphingolipids, J. Neurosci. 30 (2010) 15400–15408.
[17] T. Doyle, Z. Chen, C. Muscoli, L.M. Obeid, D. Salvemini, Intraplantar-injected ceramide in rats induces hyperalgesia through an NF-kappaB- and p38 kinase-dependent cyclooxygenase 2/prostaglandin E2 pathway, FASEB J. 25 (2011) 2782–2791.
[18] J.L. Hylden, G.L. Wilcox, Intrathecal morphine in mice. A new technique, Eur. J. Pharmacol. 67 (1980) 313–316.
[19] Z. Seltzer, R. Dubner, Y. Shir, A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury, Pain 43 (1990) 205–218.
[20] N. Kiguchi, T. Maeda, Y. Kobayashi, S. Kishioka, Up-regulation of tumor necrosis factor-alpha in spinal cord contributes to vincristine-induced mechanical allodynia in mice, Neurosci. Lett. 445 (2008) 140–143.
[21] L.E. Quintern, E.H. Schuchman, O. Levran, M. Suchi, K. Ferlinz, H. Reinke, K. Sandhoff, R.J. Desnick, Isolation of cDNA clones encoding human acid sphingomyelinase: occurrence of alternatively processed transcripts, EMBO J. 8 (1989) 2469–2473.
[22] E.H. Schuchman, M. Suchi, T. Takahashi, K. Sandhoff, R.J. Desnick, Human acid sphingomyelinase. Isolation, nucleotide sequence and expression of the full-length and alternatively spliced cDNAs, J. Biol. Chem. 266 (1991) 8531–8539.
[23] O. Levran, R.J. Desnick, E.H. Schuchman, Niemann-Pick disease: A frequent missense mutation in the acid sphingomyelinase gene of Ashkenazi Jewish type A and B patients, Proc. Natl. Acad. Sci. USA 88 (1991) 3748–3752.
[24] R. Devillard, S. Galvani, J.C. Thiers, J.L. Guenet, Y. Hannun, J. Bielawski, A. Negre-Salvayre, R. Salvayre, N. Auge, Stress-induced sphingolipid signaling: role of type-2 neutral sphingomyelinase in murine cell apoptosis and proliferation, PLoS One 5 (2010) e9826.
[25] C.J. Clarke, T.G. Truong, Y.A. Hannun, Role for neutral sphingomyelinase-2 in tumor necrosis factor alpha-stimulated expression of vascular cell adhesion molecule-1 (VCAM) and intercellular adhesion molecule-1 (ICAM) in lung epithelial cells: p38 MAPK is an upstream regulator of nSMase2, J. Biol. Chem. 282 (2007) 1384–1396.
[26] N. Kiguchi, Y. Kobayashi, S. Kishioka, Chemokines and cytokines in neuroinflammation leading to neuropathic pain, Curr. Opin. Pharmacol. (2011).
[27] L. Cao, F.Y. Tanga, J.A. Deleo, The contributing role of CD14 in toll-like receptor 4 dependent neuropathic pain, Neuroscience 158 (2009) 896–903.
[28] K. Ren, R. Torres, Role of interleukin-1beta during pain and inflammation, Brain Res. Rev. 60 (2009) 57–64.
[29] E.D. Milligan, L.R. Watkins, Pathological and protective roles of glia in chronic pain, Nat. Rev. Neurosci. 10 (2009) 23–36.
[30] U.K. Hanisch, Microglia as a source and target of cytokines, Glia 40 (2002) 140–155.
[31] R.S. Akundi, E. Candelario-Jalil, S. Hess, M. Hull, K. Lieb, P.J. Gebicke-Haerter, B.L. Fiebich, Signal transduction pathways regulating cyclooxygenase-2 in lipopolysaccharide-activated primary rat microglia, Glia 51 (2005) 199–208.
[32] J.A. Coull, S. Beggs, D. Boudreau, D. Boivin, M. Tsuda, K. Inoue, C. Gravel, M.W. Salter, Y. De Koninck, BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain, Nature 438 (2005) 1017–1021.
[33] T.A. Samad, K.A. Moore, A. Sapirstein, S. Billet, A. Allchorne, S. Poole, J.V. Bonventre, C.J. Woolf, Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity, Nature 410 (2001) 471–475.
[34] H. Hirata, H. Hibasami, T. Yoshida, M. Ogawa, M. Matsumoto, A. Morita, A. Uchida, Nerve growth factor signaling of p75 induces differentiation and ceramide-mediated apoptosis in Schwann cells cultured from degenerating nerves, Glia 36 (2001) 245–258.
[35] M.Y. Kim, C. Linardic, L. Obeid, Y. Hannun, Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation, J. Biol. Chem. 266 (1991) 484–489.
[36] L.R. Ballou, C.P. Chao, M.A. Holness, S.C. Barker, R. Raghow, Interleukin-1-mediated PGE2 production and sphingomyelin metabolism. Evidence for the regulation of cyclooxygenase gene expression by sphingosine and ceramide, J. Biol. Chem. 267 (1992) 20044–20050.
[37] T. Melcher, S. Maas, A. Herb, R. Sprengel, P.H. Seeburg, M. Higuchi, A mammalian RNA editing enzyme, Nature 379 (1996) 460–464.
[38] L.R. Ballou, S.J. Laulederkind, E.F. Rosloniec, R. Raghow, Ceramide signalling and the immune response, Biochim. Biophys. Acta 1301 (1996) 273–287.