Eur Spine J
DOI 10.1007/s00586-011-1854-y

ORIGINAL ARTICLE

Etanercept attenuates pain-related behavior following compression of the dorsal root ganglion in the rat

Kazuyuki Watanabe · Shoji Yabuki · Miho Sekiguchi · Shin-ichi Kikuchi · Shin-ichi Konno

Received: 23 February 2011 / Revised: 25 April 2011 / Accepted: 18 May 2011
© Springer-Verlag 2011

Abstract
Purpose TNFα is an inflammatory mediator related to neuropathic pain including sciatica. Much basic research suggests that anti-TNFα therapy may be useful for the treatment of sciatica. The purpose of this study was to clarify the effects of etanercept in a dorsal root ganglion (DRG) compression model.
Methods Adult male Sprague-Dawley rats (200–250 g, $n = 60$) were used. An L-shaped stainless rod was used to compress the left L5 DRG in the saline and etanercept groups. No rod was used in the sham group. In the etanercept group, 1 mg of etanercept was applied locally onto the DRG at the end of surgery. Saline was applied in the saline and sham groups. On day 3 and day 7 after surgery, the number of ED1-immunoreactive (IR) cells (macrophages) in the DRG was calculated by immunohistochemical methods ($n = 6$). In addition, double-immunofluorescence labeling for ED1 and TNFα was performed. Behavioral testing with von Frey filaments and a heat stimulator was performed ($n = 12$).
Results ED1-IR cells in the DRG significantly increased in the control group compared with the sham group ($p < 0.05$). Some ED1-IR cells were co-labeled for TNFα. In the etanercept group, decrease in mechanical threshold was significantly inhibited compared with the saline group ($p < 0.05$). Thermal hyperalgesia was observed in the control group, but in neither the sham nor etanercept group ($p < 0.05$).
Conclusion Etanercept attenuated the pain-related behavior induced by DRG compression. These findings suggest that mechanical effects on the DRG might be reduced by etanercept in addition to the effects on nucleus pulposus in lumbar disc herniation.

Keywords Sciatica · Dorsal root ganglion · Tumor necrosis factor-α · Etanercept · Macrophage

Introduction

Sciatica is a major symptom of degenerative lumbar spinal disorders such as disc herniation and spinal canal stenosis. Mechanical compression of the spinal nerve root and/or the dorsal root ganglion (DRG) is one important pathophysiological factor in such symptoms, while chemical irritation induced by nucleus pulposus or nerve injury may be another. Mechanical compression of the nerve root and/or DRG induces edema and thrombosis of nerve tissue [28], decrease in blood flow, and increase in endoneurial pressure [13], as well as pain-related behavior and hyperexcitability of DRG neurons [11, 18, 33, 39]. Compression of the DRG produces long periods of repetitive firing [10, 34]. We previously reported that compression of the DRG in rats produced pain-related behavior and structural changes in the spinal cord [38]. These findings suggest that mechanical compression of the DRG is closely related to pain. On the other hand, it has already been reported that nucleus pulposus could induce pain without any mechanical compression of the nerve and that TNFα plays a very important role in the mechanism of pain induced by nucleus pulposus [14, 19, 24–27, 29, 35]. Peripheral nerve injury induces TNFα and TNFα receptor expression in the Schwann cells in injured nerves, satellite cells, and neurons

K. Watanabe (✉) · S. Yabuki · M. Sekiguchi · S. Kikuchi · S. Konno
Department of Orthopaedic Surgery, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima, Fukushima 960-1295, Japan
e-mail: kazu-w@fmu.ac.jp

Published online: 02 June 2011

in the DRG, as well as astrocytes in the spinal cord [23, 37]. TNFα antagonists attenuate the pain-related behavior observed in experimental models of neuropathic pain including the DRG compression model [9, 30–32]. TNFα and inflammatory responses may thus play important roles in the neuropathic pain induced by nerve injury and DRG compression.

It has been reported that macrophage infiltration increased in the DRG after peripheral nerve injury and application of nucleus pulposus [7, 12, 17, 21], and that thermal hyperalgesia after chronic constriction nerve injury was reduced in mice, with inhibition of macrophage infiltration [16, 22]. These findings suggest that macrophages may play important roles in the neuropathic pain that occurs following nerve injury.

Etanercept is a soluble TNFα receptor-Fc protein and neutralizes TNFα [20]. Macrophages in the synovium of joints affected by rheumatoid arthritis decrease in number due to apoptosis following anti-TNFα therapy [2]. In addition, TNFα inhibitor reduces infiltration of macrophages with myeloid-related proteins 8 and 14 from the peripheral circulation [5]. A previous experimental study suggested that etanercept reduced neuropathic pain [32]. Etanercept may thus reduce macrophage infiltration into nerve tissue, and alleviate neuropathic pain.

The objective of this study was, first, to observe the changes in macrophages and TNFα in compressed DRG. Secondly, we investigated the effects of etanercept on pain-related behavior following DRG compression using behavioral testing.

Materials and methods

Seventy-two adult male Sprague-Dawley rats (body weight 220–300 g) were used in this study. All animal experiments conformed to the regulations of the Animal Research Committee of Fukushima Medical University, which accorded with the Guidelines on Animal Experiments of Fukushima Medical University and Japanese Government Animal Protection and Management Law (No. 105).

Surgical procedure

Dorsal root ganglion compression was induced as described in our previous report [38]. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The left L5 lamina was exposed and a drill hole (2.0 × 1.5 mm) was made in the lamina using an electric drill. In the compression group ($n = 48$), an L-shaped stainless steel rod with a diameter of 0.5 mm and a side length of 2 mm was inserted into the drill hole distally along the DRG and nerve root. The DRG and proximal part of the nerve root were compressed with the side of the L-shaped rod (Fig. 1). In the sham group ($n = 24$), only the drill hole was made without insertion of the rod. The compression group was divided into two subgroups: a saline group (non-treatment group, $n = 24$) and an etanercept group (treatment group, $n = 24$). In the etanercept group, a piece of Spongel (Yamanouchi Pharmaceutical, Tokyo, Japan) containing 1 mg/20 μl etanercept (Wyeth K.K, Tokyo, Japan) was applied to the left L5 DRG at the end of surgery. The amount of etanercept was decided according to a previous report [30]. In the saline group, a Spongel containing 20 μl saline was applied instead of etanercept to evaluate the effects of etanercept on DRG compression. In the sham group, Spongel with saline was also applied. We set the sham group as no compression with no treatment for comparison with the saline group (DRG compression with no treatment) in order to determine the effects of DRG compression.

Immunohistochemistry for ED1

ED1 was selected to observe macrophages in the DRG according to previous reports [6, 12, 17, 21]. Our

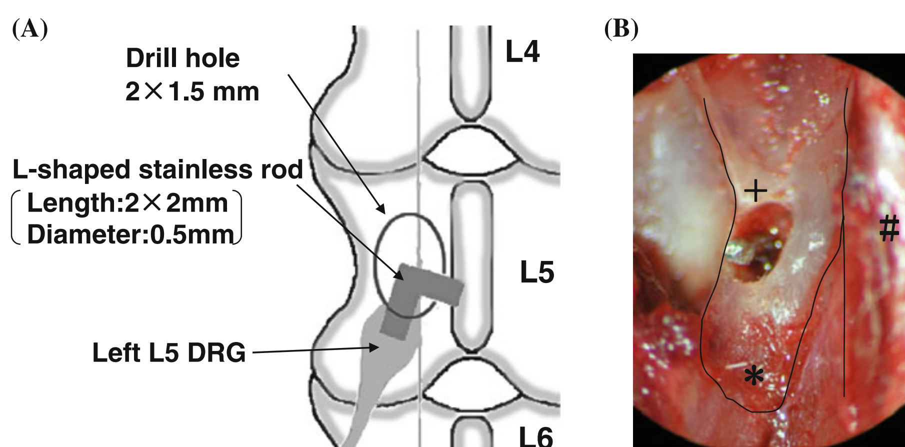


Fig. 1 The schematic figure (**a**) and photo (**b**) show the surgical procedure for inducing compression of the left L5 DRG. An L-shaped stainless steel rod was inserted into the drill hole distally along the nerve root and DRG. *Plus symbol* drill hole with inserted rod, *asterisk* left L5 inferior articular process, *hash symbol* L5 spinous process

preliminary study showed ED1-IR cells were increased at day 3 and day 7 but not day 14 or 28. Therefore, we observed ED1-IR cells at day 3 and day 7. Thirty-six rats were used in this experiment (each group: $n = 12$). At 3 and 7 days after surgery, six rats each were deeply anaesthetized with inhalation of ether and subjected to intracardiac perfusion with 200 ml saline followed by 300 ml of paraformaldehyde in 0.1 M PBS. Left L5 DRGs were then removed and postfixed overnight in the same fixative. Tissue was embedded in paraffin and cut into transverse 6 μm sections. The sections were mounted on slides, deparaffinized with xylene, rehydrated in a graded ethanol series, and processed immunohistochemically. Tissue sections were preincubated in 0.3% H_2O_2 in PBS for 10 min and then washed in PBS. The tissue sections were incubated for 30 min at room temperature in a blocking solution of 1.5% normal horse serum in 0.1 M PBS and then incubated overnight at 4°C in mouse anti-monocyte/macrophage antibody (ED1; Chemicon, Temecula, CA, USA) diluted 1:300. The incubated sections were washed in PBS and incubated in biotinylated anti-mouse IgG antibody diluted 1:200 (Vector Laboratories, Burlingame, CA, USA) for 60 min at room temperature, and then washed in PBS and incubated for 30 min in avidin–biotin–peroxidase complex (ABC; Vector Laboratories). Finally, the sections were washed in PBS and developed in 0.02% 3,3′-diaminobenzidine dihydrochloride (DAB). After staining, tissue sections were coverslipped and examined with a light microscope. The investigator counting the ED1-IR cells was blind to experimental conditions. ED1-IR cells were analyzed in sections from the central one-third of the DRG. The total number of ED1-IR cells in each whole section was counted using imaging analysis software. Three randomly selected sections were examined per animal, and the mean for each animal was obtained. Results are expressed as the mean ± standard error for each experimental group.

Double immunofluorescence

Six micrometre-thick paraffin sections were prepared at days 3 and 7 as described above, and pretreated with 0.1% trypsin at 37°C for 20 min to enhance immunoreactivity. After blocking with 2% gelatin, double-label immunofluorescence was performed by incubating sections with primary antibody including gout anti-TNF (1:50) (R&D Systems Inc., Minneapolis, MN, USA) and ED1 (1:300) (Chemicon International) overnight at 4°C. The slides were then incubated for 1 h with a secondary antibody labeled with Alexa Fluor 488 (1:200) (green) or Alexa Fluor 555 (1:200) (red) (Molecular Probes, Carlsbad, CA, USA). Fluorescence staining was examined using an Olympus Optical BX50 fluorescence microscope equipped with Axio Vision imaging software (Carl Zeiss, Göttingen, Germany).

Behavioral testing

In the saline, etanercept, and sham groups, 12 rats each were used to examine thresholds of withdrawal to mechanical and thermal stimulation at 1 day before surgery as a baseline, and 3, 7, 14, 21, and 28 days after surgery. For measurement of mechanical withdrawal thresholds, rats were placed in a transparent plastic box with a wire netting floor and allowed to acclimate for 15 min. The mid-plantar surface of the left hindpaw was stimulated using von Frey filaments capable of exerting bending forces of 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, 15.1, and 28.8 g. We performed one trial for each filament using an up-down method as described in our previous report [38]. In total, six or more trials were performed for each rat. The force of stimulation that caused a brisk withdrawal in 50% of trials was calculated using Chaplan's method [3]. Results were expressed as the mean ± standard error (SE) of 50% withdrawal threshold.

For investigation of nociceptive responses to thermal stimulation, the rats were placed in a clear plastic chamber with a glass floor and were allowed to acclimate for 10 min before testing. A radiant heat source (Ugo Basille, Italy) was positioned under the glass floor directly beneath the hindpaw. The time of foot withdrawal upon thermal stimulation was measured as described in Hargreaves' report [8]. The hindpaw on each side was alternately tested at interstimulus intervals of 10 min, twice for each hindpaw. A difference score (DS) was calculated by subtracting the average latency of the control side from the average latency of the operated side [8]. Results are expressed as the mean ± SE of DS.

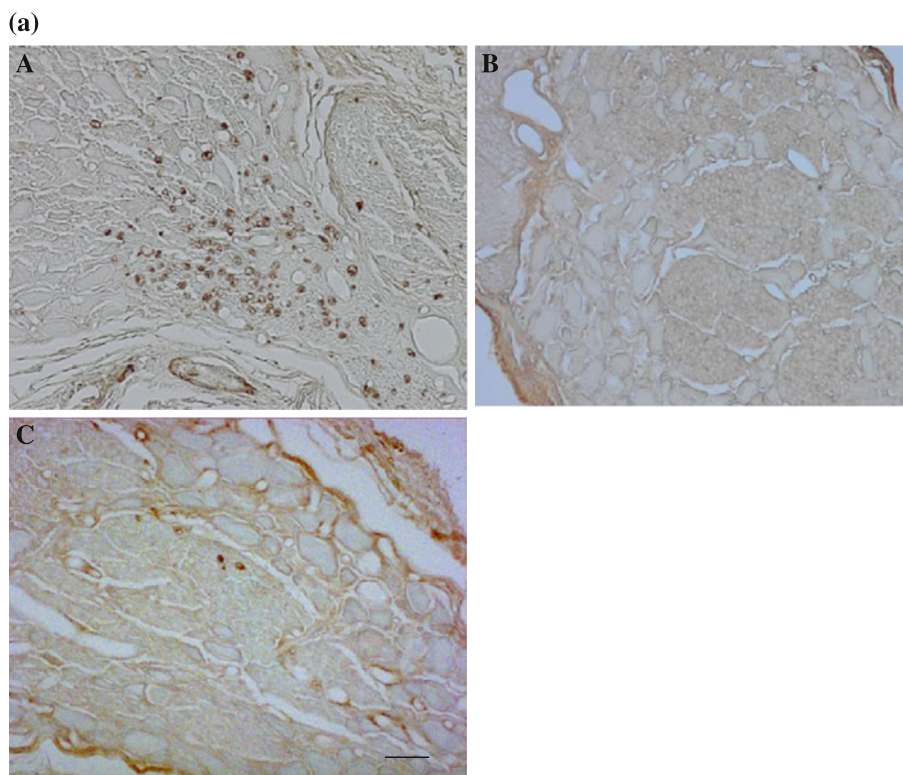
Statistical analysis

The Kruskal–Wallis test was used, followed by Mann–Whitney's U test with Bonferroni correction, with p values <0.05 considered significant.

Results

Expression of ED1-IR cells (Fig. 2a, b)

At day 3, there were no significant differences among the three groups. However, at day 7, marked increase in the number of ED1-IR cells was observed in the saline group, with a few ED1-IR cells observed in the sham group. At day 7, the number of ED1-IR cells was higher in the etanercept group than in the sham group ($p < 0.05$), but



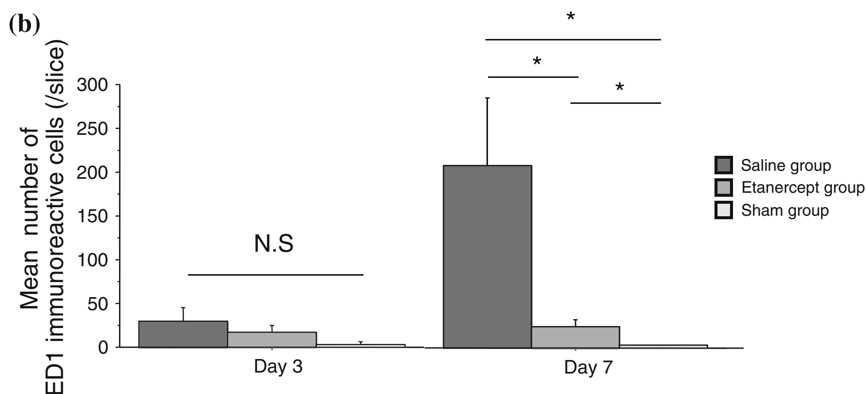


Fig. 2 **A** Photomicrographs demonstrating ED1-IR cells in DRG at day 7. *a* Saline group, *b* sham group, and *c* etanercept group. These sections are transverse sections from one third of the central portion in DRG. In the saline group, many ED1-IR cells were observed in the DRG tissue, while only a few ED1-IR cells were observed in the sham and etanercept groups. *Scale bar* 50 µm. **B** Graph of numbers of ED1-IR cells in DRG. Results are the mean ± standard error of number of ED1-IR cells. At day 3, there were no significant differences in number of ED1-IR cells among the three groups. At day 7, marked increase in number of ED1-IR cells was observed in the saline group compared with the sham and etanercept groups ($p < 0.05$)

significantly less than that in the saline group ($p < 0.05$). These findings suggested that compression of the DRG induced increase in number of ED1-IR cells in the compressed DRG which was reduced by etanercept.

Double immunofluorescence for ED1 and TNFα (Fig. 3)

Photomicrograph of DRG at day 7 demonstrating immunoreactivity for ED1 (green) and TNFα (red) were presented. In the saline group, both neurons and the interneuronal regions exhibited immunoreactivity for TNFα, and co-localization for ED1 was observed. On the other hand, the sham and etanercept groups exhibited less immunoreactivity for TNFα than the saline group. There were few ED1-IR cells in the sham and etanercept groups. At day 3, there were few ED1-IR cells exhibiting co-localization for TNF in each group (data not shown). These findings suggested that DRG compression induced increase in ED1-IR cells co-localized with TNFα which was inhibited by etanercept.

Mechanical withdrawal threshold (Fig. 4)

In the saline group, threshold decreased after DRG compression, and significant differences compared with the sham group were observed at days 7, 21, and 28. In the etanercept group, significant differences compared with

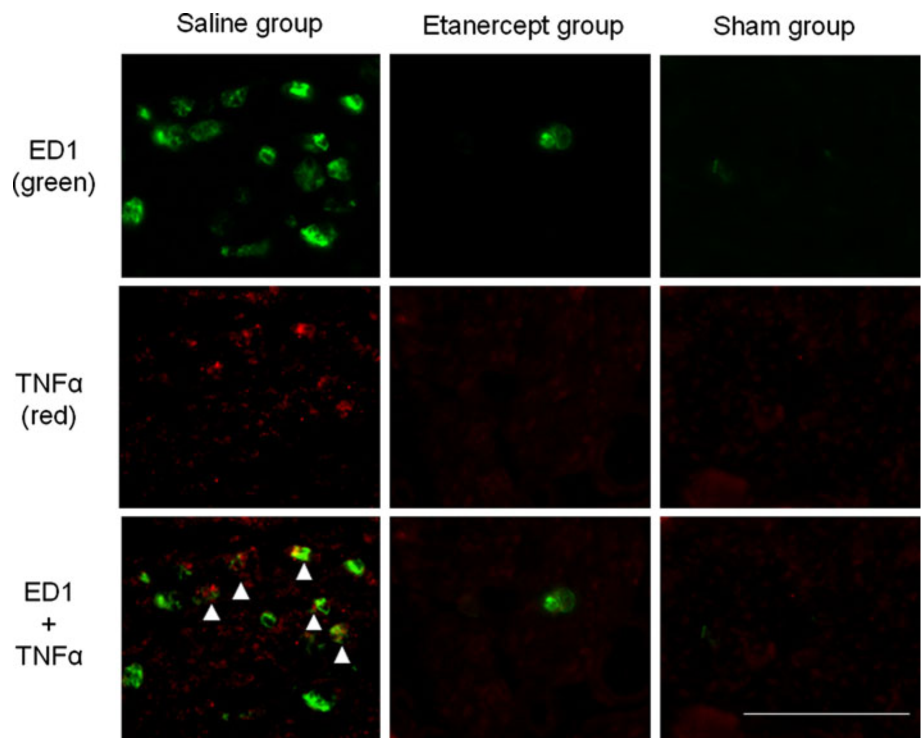


Fig. 3 Photomicrograph of DRG at day 7 demonstrating immunoreactivity for ED1 (*green*) and TNFα (*red*). In the saline group, some ED1-IR cells exhibited co-localization of staining for TNFα (*triangles*), with few ED1-IR cells in the sham and etanercept groups. *Scale bar* 50 μm

the sham group were observed only at day 28, while the threshold decreased after operation. However, the etanercept group exhibited significant increase in threshold compared with the saline group at day 7 ($p < 0.05$). These findings indicated that etanercept attenuated the pain behavior induced by DRG compression.

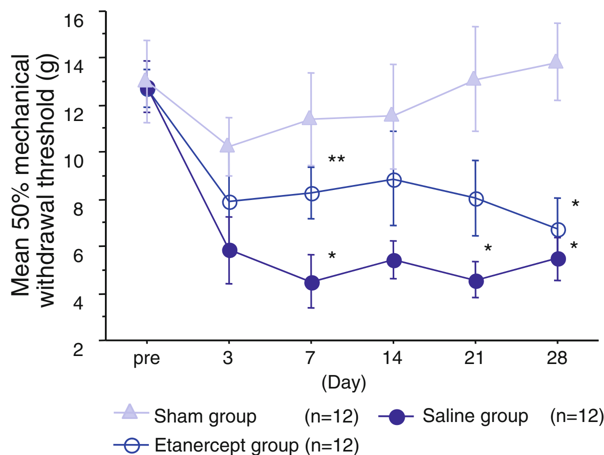


Fig. 4 Time course of change in mechanical sensitivity. Results are the mean ± standard error of 50% withdrawal threshold. There were significant differences in threshold between the saline and sham groups at days 7, 21, and 28, and between the etanercept and sham groups at day 28 alone (*$p < 0.05$). The etanercept group exhibited significant differences in threshold from the saline group at day 7 (**$p < 0.05$). These findings suggested that DRG compression induced mechanical hypersensitivity from day 7 to day 28 which was attenuated by etanercept at day 7. The effect of etanercept appeared to disappear at day 28

Thermal withdrawal threshold (Fig. 5)

The difference score (DS) in the saline group was decreased after operation from day 3 to day 14, while the sham and etanercept groups exhibited no marked changes in DS from the baseline of zero. The saline group exhibited significant decrease in DS compared with the sham group as well as the etanercept group at day 7 ($p < 0.05$). These findings suggested that DRG compression induced hypersensitivity to heat stimulation that was attenuated by etanercept.

Discussion

Our findings showed that compression of the DRG induced increase in number of ED1-IR cells in the compressed DRG and expression of TNFα. In addition, etanercept, when applied directly to the DRG, attenuated the pain-related behaviors induced by DRG compression and inhibited the increase in ED1-IR cells. One previous study showed that in rats with chronic constriction injury (CCI), endoneurial TNF was increased within 12 hours after surgery [7]. In addition, several studies showed that anti-TNFα therapy for animals with nerve injury including DRG compression was effective only in the early stage of pain-related behavior [9, 30, 31]. These findings suggest that TNFα is related to the initiation of neuropathic pain. TNFα thus also appeared to be important in initiation of behavioral changes in our

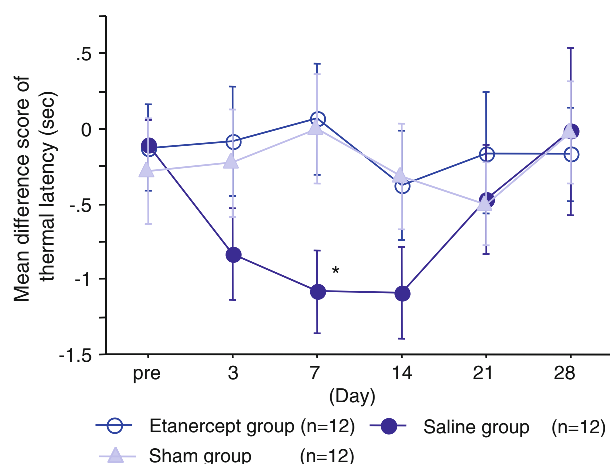


Fig. 5 Time course of change in difference score (DS) as a measure of thermal latency. Results are the mean ± standard error of response time. In the saline group, DS decreased after DRG compression and was minimum at day 7 after surgery, while DS in the sham group did not decrease. There were significant differences between the saline group and sham group at day 7. On the other hand, in the etanercept group, DS did not significantly decrease during the observation period. There was a significant difference in DS between the etanercept and saline groups at day 7 (*$p < 0.05$). These findings suggested that thermal hypersensitivity was induced by DRG compression at day 7, which was attenuated by etanercept

animal model, and etanercept appeared to inhibit behavioral changes after DRG compression. However, in our model, development of behavioral changes was slower than in other neuropathic pain models. About one week is required to reach maximum effect in our model. Therefore, the time course of change in TNFα appeared to be slower than in other neuropathic pain models. This could explain why the effect of etanercept was observed only 7 days postoperatively. Interestingly, mechanical hypersensitivity tended to be reduced until day 21 by preventive application of etanercept. When etanercept is administered to humans via subcutaneous injection, its half-life is 4-5 days. However, the pharmacokinetics of etanercept applied via local injection in the rat have not been reported. The duration of therapeutic effect may have extended beyond the time of clearance of etanercept in this study.

Peripheral nerve injury induces inflammatory responses such as infiltration of inflammatory cells into the injured nerve tissue. In our study, the finding of increase in macrophages in the compressed DRG indicated that inflammatory response occurred after compression of the DRG, and that etanercept inhibited the inflammatory cascade in the compressed DRG by blocking TNFα. In addition, macrophages have other important functions such as phagocytosis. WLD mice, in which delayed Wallerian degeneration is observed after peripheral nerve injury, exhibit inhibition of neuropathic pain and macrophage infiltration [22]. Mice with knockout of chemokine receptor 2 (CCR2), an important factor inducing infiltration of macrophages, exhibited no neuropathic pain after sciatic nerve ligation [1]. These previous findings suggest that macrophages have important functions in the mechanisms of neuropathic pain. There might thus be a relationship between reduction of infiltration of macrophages into the compressed DRG and improvement of pain-related behaviors by etanercept.

Previous basic research has shown that TNFα is closely related to the pain induced by nucleus pulposus [14, 19, 24–27, 29, 35], and anti-TNFα therapy has correspondingly been focused upon as a new treatment for lumbar disc herniation. The findings of the present study indicate that TNFα is also related to the pain induced by mechanical compression of the DRG. This may support the use of anti-TNFα therapy for the sciatica induced by lumbar disc herniation and lumbar spinal canal stenosis.

Regarding clinical use of anti-TNFα therapy, some groups have reported the efficacy of etanercept in treating low back pain and sciatica [4, 36]. Side-effects of these anti-TNF therapies are a significant problem, although no side-effects were reported [4, 36]. Not only infections due to immunosuppression but also demyelination could be a severe problem in radiculopathy with motor dysfunction. However, one experimental study showed that etanercept enhanced axonal regeneration in injured sciatic nerve [15]. Therefore, another study using different animal models which shows clear motor dysfunction is needed to examine the effect of etanercept on demyelination. Unsolved problems also remain concerning the route and timing of application of etanercept, as well as its dosage and selection of patients for treatment with it. Our findings showed that direct application of etanercept attenuated pain-related behavior and inflammatory reaction in DRG compressed rat. Therefore, direct application of etanercept to the nerve, such as nerve root infiltration, might be effective clinically. One clinical study indicated that transforaminal injection of etanercept could reduce sciatica [4]. In addition, the effective dosage with direct application is expected to be lower than that for subcutaneous injection, and this could reduce side effects of etanercept. In future studies, comparison of subcutaneous and direct application is needed.

In conclusion, etanercept, a soluble TNFα receptor, attenuated the pain-related behavior induced by DRG compression and inhibited increase in number of macrophages in compressed DRG. These findings suggest that TNF inhibition might not only modulate nucleus pulposus-induced effects but also the mechanical effects in sciatica induced by lumbar disc herniation. This suggests the usefulness of anti-TNFα therapy as an option for conservative treatment of the sciatica induced by lumbar disc herniation and spinal canal stenosis.

Acknowledgments The authors thank Satoshi Sato and Rie Shibuya for expert technical assistance.

Conflict of interest No funds were received in support of this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

References

1. Abbadie C, Lindia JA, Cumiskey AM et al (2003) Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci 100:7947–7952
2. Catrina AI, Trollmo C, af Klint E et al (2005) Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joins: extended report. Arthritis Rheum 52:61–72
3. Chaplan SR, Bach FW, Pogrel JW et al (1994) Quantitative assessment of tactile allodynia in rat paw. J neurosci methods 53:55–63
4. Cohen SP, Bogduk N, Dragovich A et al (2009) Randomized, double-blind, placebo-controlled, dose-response, and preclinical safety study of transforaminal epidural etanercept for the treatment of sciatica. Anesthesiology 110:1116–1126
5. De Rycke L, Basten D, Foell D et al (2005) Differential expression and response to anti-TNF alpha treatment of infiltrating versus resident tissue macrophage subset in autoimmune arthritis. J Pathol 206:17–27
6. Fenzi F, Benedetti MD, Moretto G et al (2001) Glial cell and macrophage reactions in rat spinal ganglion after peripheral nerve lesions: an immunocytochemical and morphometric study. Arch Ital Biol 139:357–365
7. George A, Marziniak M, Schafers M et al (2000) Thalidomide treatment in chronic constrictive neuropathy decreases endoneurial tumor necrosis factor-alpha, increases interleukin-10 and has longterm effects on spinal cord dorsal horn met-enkephalin. Pain 88:267–275
8. Hargreaves K, Dubner R, Brown F et al (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88
9. Homma Y, Brull SJ, Zhang JM (2002) A comparison of chronic pain behavior following local application of tumor necrosis factor alpha to the normal and mechanically compressed lumbar ganglia in the rat. Pain 95:239–246
10. Howe JF, Loser JD, Calvin WH (1977) Mechanosensitivity of dorsal root ganglioa and chronically injured axons: a physiological basis for the radicular pain of nerve root compression. Pain 3:25–41
11. Hu SJ, Xing JL (1998) An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. Pain 77:15–23
12. Hu P, Mclachlan EM (2002) Macrophage and lymphocyte invasion of dorsal root ganglion after peripheral nerve lesions in the rat. Neuroscience 112:23–38
13. Igarashi T, Yabuki S, Kikuchi S et al (2005) Effect of acute nerve root compression on endoneural fluid pressure and blood flow in rat dorsal root ganglia. J Orthop Res 23:420–424
14. Igarashi T, Kikuchi S, Shubayeb V et al (2000) Exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Spine 25:2975–2980
15. Kato K, Liu H, Kikuchi S, Myers RR, Shubayev VI (2010) Immediate anti-tumor necrosis factor-alpha (etanercept) therapy enhances axonal regeneration after sciatic nerve crush. J Neurosci Res 88:360–368
16. Liu T, Van Rooijen N, Tracey DJ (2000) Depletion of macrophages reduces axonal degeneration and hyperalgesia following nerve injury. Pain 86:25–32
17. Lu X, Richardson PM (1993) Responses of macrophages in rat dorsal root ganglia following peripheral nerve injury. J Neurocytol 22:334–341
18. Ma C, Lamotte RH (2005) Enhanced excitability of dissociatied primary sensory after chronic compression of the dorsal root ganglion in the rat. Pain 113:106–112
19. Miyoshi S, Sekiguchi M, Konno S et al (2011) Increased expression of vascular endothelial growth factor protein in dorsal root ganglion exposed to nucleus pulposus on the nerve root in rats. Spine 36:E1–E6
20. Mohler KM, Torrance DS, Smith CA et al (1993) Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagosists. J Immunol 151:1548–1561
21. Murata Y, Rydevik B, Takahashi K et al (2004) Macrophage appearance in the epineurium and endoneurium of dorsal root ganglion exposed nucleus pulposus. J Peripher Nerv Syst 9:158–164
22. Myers RR, Heckman HM, Rodriguez M (1996) Reduced hyperalgesia in nerve-injured WLD mice: relationship to nerve fiber phagocytosis, axonal degeneration, and regeneration in normal mice. Exp Neurol 141:94–101
23. Ohtori S, Takahashi K, Moriya H et al (2004) TNF-α and TNF-α receptor type 1 upregulation in glia and neurons after peripheral nerve injury. Spine 29:1082–1088
24. Olmarker K, Rydevik B (2001) Selective inhibition of tumor necrosis factor-alpha prevents nucleus pulposus-induced thrombus formation, intraneural edema, and reduction of nerve conduction velocity: possible implications for future pharmacologic treatment strategies of sciatica. Spine 26:863–869
25. Olmarker K, Nutu M, Storkson R (2003) Changes in spontaneous behavior in rats exposed to experimental disc herniation are blocked by selective TNF-Alpha inhibition. Spine 28:1653–1642
26. Onda A, Yabuki S, Kikuchi S (2003) Effect of neutralizing antibody to tumor necrosis factor-alpha on nucleus pulposus-induced abnormal nociresponses in rat dorsal horn neurons. Spine 28:967–972
27. Otoshi K, Kikuchi S, Konno S et al (2010) The reactions of glial cells and endoneurial macrophages in the dorsal root ganglion and their contribution to pain-related behavior after application of nucleus pulposus onto the nerve root in rats. Spine 35:10–17
28. Rydevik BL, Myers RR, Powell HC (1989) Pressure increase in the dorsal root ganglion following mechanical compression. Closed compartment syndrome in nerve roots. Spine 14:674–678
29. Sasaki N, Sekiguchi M, Kikuchi S et al (2011) Effects of asialo-erythropoietin on pain-related behavior and expression of phosphorylated-p38 map kinase and tumor necrosis factor-alpha induced by application of autologous nucleus pulposus on nerve root in rat. Spine 36:E86–E94
30. Schafers M, Svensson CI, Sommer C, Sorkin LS (2003) Tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons. J neurosci 23:2517–2521
31. Sommer C, Marziniak M, Myers RR (1998) The effect of thalidomide treatment on vascular pathology and hyperalgesia caused by chronic constriction injury of rat nerve. Pain 74:83–91
32. Sommer C, Schafers M, Marziniak M, Toyka KV (2001) Etanercept reduces hyperalgesia in experimental painful neuropathy. J Peripher Nerv Syst 6:67–72
33. Song XJ, Hu AJ, Greenquist KW, Zhang J-M, LaMotte RH (1999) Mechanical and thermal hyperalgesia and ectopic

neuronal discharge after chronic compression of dorsal root ganglion. J Neurophysiol 82:3347–3358
34. Sugawara O, Atsuta Y, Iwahara T et al (1996) The effect of mechanical compression and hypoxia on nerve root and dorsal root ganglia: an analysis of ectopic firing using an in vitro model. Spine 21:2089–2094
35. Tachihara H, Sekiguchi M, Kikuchi S et al (2008) Do corticosteroids produce additional benefit in nerve root infiltration for lumbar disc herniation? Spine 33:743–747
36. Tobinick E (2010) Perispinal etanercept: a new therapeutic paradigm in neurology. Expert Rev Neurother 10:985–1002
37. Wagner R, Myers RR (1996) Schwann cells produce tumor necrosis factor alpha: expression in injured and non-injured nerves. Neuroscience 73:625–629
38. Watanabe K, Konno S, Sekiguchi M (2007) Increase of 200kD-neurofilament immunoreactive afferents in the substantia gelatinosa in allodynic rats induced by compression of the dorsal root ganglion. Spine 32:1265–1271
39. Zhang JM, Song XJ, LaMotte RH (1999) Enhanced excitability of sensory neurons in rats with cutaneous hyperalgesia produced by chronic compression of the dorsal root ganglion. J Neurophysiol 82:3359–3366