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Effect of Minocycline on Activation of Glia and Nuclear Factor kappa B in an Animal Nerve Injury Model

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Glial cells are activated in neuropathy and play a key role in hyperalgesia and allodynia. This study was performed to determine whether minocycline could attenuate heat hyperalgesia and mechanical allodynia, and how glial cell activation and nuclear factor kappa B (NF-kappaB) were regulated by minocycline in a model of chronic constriction of sciatic nerve (CCI). When minocycline (50 mg/kg, oral) was daily administered from 1 day before to 9 days after ligation, heat hyperalgesia and mechanical allodynia were attenuated. Furthermore, when minocycline treatment was initiated 1 or 3 days after ligation, attenuation of the hypersensitive behavior was still robust. However, the effect of attenuation was less when minocycline was started from day 5. In order to elucidate the mechanism of pain attenuation by minocycline, we examined the changes of glia and NF-kappaB, and found that attenuated hyperalgesia and allodynia by minocycline was accompanied by reduced microglial activation. Furthermore, the number of NF-kappaB immunoreactive cells increased after CCI treatment and this increase was attenuated by minocycline. We also observed translocation of NF-kappaB into the nuclei of activated glial cells. These results suggest that minocycline inhibits activation of glial cells and NF-kappaB, thereby attenuating the development of behavioral hypersensitivity to stimuli.

Key Words: Minocycline, Glia, NF-kappaB, Spinal cord, Neuropathy

INTRODUCTION

Chronic pain can occur after peripheral nerve injury, infection, or inflammation. Under such neuropathic pain conditions, sensory processing in the affected body region becomes abnormal. Currently available drugs largely fail to control such pain. To elucidate pathogenic factors and develop therapeutic strategies aimed at halting its progression, many researchers have been investigating the neuropathology of neuropathic pain. Aside from the neuronal component, it seems that the non-neuronal cells of the central nervous system (CNS) such as glial cells also have a role in the initiation and maintenance of persistent pain states (DeLeo & Yezierski, 2001; Watkins et al, 2001a, b).

Microglia have neurotrophic and neurotoxic effects that are important in the CNS inflammatory responses, neuronal repair and regeneration after injury (Kreutzberg, 1990; Moore & Thanos, 1996; Ye & Johnson, 1999). Peripheral nerve injury stimulates microglial proliferation in the CNS (Aldskogius & Kozlova, 1998). Moreover, microglial cells may be important in the cascade of events that lead to thermal hyperalgesia (Meller et al, 1994; Watkins et al, 1997). However, there are still conflicting reports on whether the development of neuropathic pain after a peripheral nerve injury is correlated with microglial activation (Colburn et al, 1997; Coyle, 1998). Astrocytic activation is common in the CNS following peripheral nerve damage or CNS injury (Gilmore et al, 1990; Garrison et al, 1991; Eriksson et al, 1997; Aldskogius & Kozlova, 1998), and hypertrophy of astrocytes is accompanied by an upregulation of glial fibrillary acid protein (GFAP, Garrison et al, 1991; Aldskogius & Kozlova, 1998). Glia has recently been implicated in the cascade of events that lead to the development of neuropathic pain (Colburn et al, 1999; Coyle, 1998). Although astrocytic responses have been correlated with pain behaviors (Garrison et al, 1991; Colburn et al, 1997), the exact relationship between astrocytic responses in the dorsal horn and pain behaviors is still unclear.

Nuclear factor kappa B (NF-kappaB) was originally identified in immune cells as a regulator gene in the process of immune response and inflammation (Baeuerle & Baltimore, 1996; Barnes & Karin, 1997). NF-kappaB's role as a critical regulator of cytokine-inducible gene expression has been recognized by the fact that many of the pro-inflammatory cytokine genes, including interleukin (IL)-1alpha, IL-6 and tumor necrosis factor (TNF)-alpha, possess NF-kappaB binding sites. When unaffected by inflammation, NF-kappaB is located in cytoplasm and inactivated by inhibitory protein of NF-kappaB (I-kappaB). However, when cells are exposed to noxious stimuli, I-kappaB kinase phosphorylates and degrades I-kappaB, and I-kappaB degradation frees NF-kappaB to enter

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ABBREVIATIONS: NF-kappaB, nuclear factor kappa B; CCI, chronic constriction of sciatic nerve; PWT, paw-withdrawal threshold; PWL, paw withdrawal latency; MAP-2, microtubule associated protein 2; GFAP, glial fibrillary acidic protein

nucleus. Recently, NF-kappaB has attracted a considerable amount of attention as a key signaling element in glial and neuronal cell function (Kaltschmidt et al, 1994; O'Neill & Kaltschmidt, 1997), and the activation of NF-kappaB has been detected in animal models of traumatic spinal cord injury (Bethea et al, 1998) and neuropathic pain (Ma & Bisby, 1998).

Minocycline, a semisynthetic second-generation tetracycline, is an antibiotic that possesses superior penetration into the CNS via the brain-blood barrier (Aronson, 1980). Minocycline has emerged as a potent inhibitor of microglial activation (Amin et al, 1996; Tikka & Koistinaho, 2001; Tikka et al, 2001a, b), and has proved to be an effective neuroprotective agent in experimental brain ischemia (Yrjanheikki et al, 1998), in the R6/2 mouse model of Huntington's disease (Chen et al, 2000), in traumatic brain injury (Sanchez Mejia et al, 2001), and in the mouse model of Parkinson's disease (Wu et al, 2002).

This study was undertaken to elucidate the effect of minocycline on neuropathic pain, activation of glial cells and NF-kappaB.

METHODS

Ligation of sciatic nerve

Sprague-Dawley male rats, weighing 175 to 200 g at the start of surgery, were used. The rats were housed in cages at room temperature under a 12/12 hours non-light/dark cycle with free access to food and water. The animals were allowed to acclimatize for 1 week before the experiments. Behavioral studies were carried out in a dedicated room. The Institutional Animal Care and Use Committee at our university approved all the procedures done in this study. Efforts were made to limit distress and to use the minimum number of animals necessary to achieve statistical significance, according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983). Unilateral sciatic nerve injury was produced under deep anesthesia with intramuscular injection of mixture of ketamin (60 mg/kg) and xylazine (7 mg/kg), as described by Bennett & Xie (1988). The common sciatic nerve was exposed and freed at mid thigh level. Four ligatures (Ethicon 4.0 plain gut) were tied loosely around the nerve with about 1-mm spacing. Great care was taken to tie the ligatures, such that the nerve was barely constricted. The muscle and skin was closed in layers with 3-0 silk sutures.

Behavioral assessments

1) von Frey filaments were used to assess the sensitivity of the skin to tactile stimulation. Each rat was placed under a transparent dome on a plastic mesh floor and each filament was applied perpendicularly to the plantar surface of the left and right hind paw of each animal. Increasing strengths of von Frey filaments were sequentially applied. Each filament was applied 5 times, and it was considered positive when rat showed responses to stimuli more than 3 times. The minimum paw-withdrawal threshold (PWT), defined as the minimum gram strength eliciting withdrawal from pressure, was recorded for each hind limb. The stimuli consisted of a set of 0.008 (1), 0.02 (2), 0.04 (3), 0.07 (4), 0.16 (5), 0.4 (6), 0.6 (7), 1.0 (8), 1.4 (9), 2.0 (10), 4.0 (11), 6.0 (12), 8.0 (13), 10.0 (14), and 15.0 g (15) of von Frey filaments (model: NC12775, North Coast medical, Inc., CA), and the scores in the parentheses were given arbitrarily. 2) The response to a heat stimulus was tested with the "Plantar Test" device from Ugo Basile (Varese, Italy). Rats were placed in bottomless, clear, rectangular Plexiglas chambers on a glass shelf. A movable radiant heat source from a halogen lamp located below the animal was focused on the hind paw, thereby gradually increasing the skin temperature at that spot. The time taken by the animal to withdraw the leg (paw withdrawal latency, PWL) was automatically measured by the device with a minimal time of 0.5 sec and a maximal cutoff at 20 sec.

Both PWT and PWL measurements were performed as follows. In a given session, sham and nerve-injured animals were tested randomly. For each animal, both hind limb paws (intact and operated) were tested alternately at 5 min intervals, and the average value of five consecutive tests for each leg was recorded. The difference between intact and operated legs was considered to reflect the degree of sensitivity. On day 0 (presurgery), each rat's sensitivity to stimuli was determined by recording the values from the both hind paws. Tests were repeated again in each rat at 1, 4, 7, and 10 days following ligation. Behavioral results presented in this paper are based on 10 rats per each group. Immediately after behavioral testing, the animals were sacrificed and used for tissue collection.

Minocycline treatment

Minocycline (50 mg/kg, Sigma) or saline was administered orally. In one group, treatment was initiated 1 d before surgery and continued daily to day 9 after surgery (group G1). In other groups, treatment started from day 1 (group G2), 3 (group G3) and 5 (group G4) following surgery.

Immunohistochemistry

After behavioral testing, rats were euthanized by ether overdose. Animals were perfused with 50 ml of normal saline and then with 200 ml of 4% phosphate-buffered paraformaldehyde. After the vertebral column was removed, spinal cord (L4-L5) was isolated, embedded in paraffin, and cut at 6 μm thickness. After deparaffinization and antigen retrieval with microwaving in 10 mM citric acid solution, sections were treated for endogenous peroxidases with 0.03% H_2O_2 and blocked in 5% normal serum, and then incubated with the primary antibody, followed by the secondary antibody (1 : 200, Vector Laboratories). Antibodies were detected by using the Elite Vectastain ABC kit (Vector) and colorized, using 3, 3'-diaminobenzidine peroxidase substrate kit (Vector). Anti-MAP-2 (1 : 500, Sigma), anti-GFAP (1 : 250, PharMingen), ED-1 (1 : 250, Bachem), and anti-NF-kappaB p65 (1 : 250, Santa Cruz) were used as the primary antibody.

Statistical analysis

Data are given as means$\pm$standard error of the mean (SE). Comparison between experimental groups were tested, using a Kruskal-Wallis one way analysis of variance of ranks followed by a Dunn's method for pairwise multiple comparisons or one way analysis of variance followed by Student-Newman-Keuls tests. We used Sigmastat software (SPSS). A probability value of less than 0.05 was considered

to be statistically significant.

RESULTS

Behavior and glial activation following sciatic nerve ligation

Before sciatic nerve ligation, all groups of animals exhibited comparable baseline thresholds to stimuli. PWL of the left hind paw in response to the radiant hear stimulation decreased significantly from the post-surgery day 1 and maintained until day 10 (Fig. 1A). PWT before sciatic nerve ligation was around 8 g (or score 12), and threshold decreased gradually and significantly from day 4 to post-ligation day 10 (Fig. 1B). Activated microglia was almost absent in the normal spinal cord, but the number significantly increased at 4 days following nerve ligation and maintained the increased number even at post-ligation day 10 (Fig. 1C and Fig. 3B). In the normal spinal cord, we could detect a small number of astrocytes with fine branches and weak GFAP staining. Following sciatic nerve ligation, the numbers of GFAP positive cells increased (Fig. 1D) and most of the astrocytes had longer branches and bigger bodies than those in the normal spinal cord (Fig. 3E).

Effect of minocycline

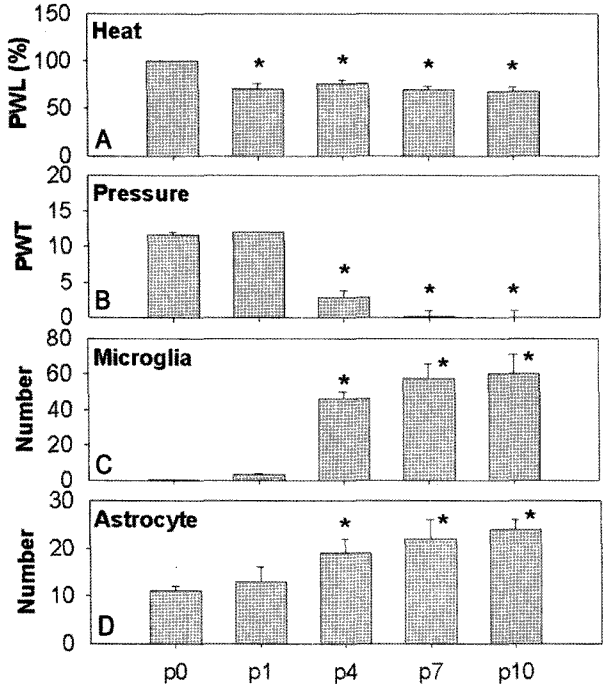

Fig. 1. Behavioral responses to thermal (A) and mechanical stimuli (B) and numbers of microglia (C) and astrocytes (D) in the ipsilateral spinal cord before (p0) and 1 day (p1), 4 days (p4), 7 days (p7) and 10 days (p10), following sciatic nerve ligation. Responses to heat (paw withdrawal latency, PWL) and mechanical stimuli (minimum paw-withdrawal threshold, PWT) were measured. Stimuli were applied to the plantar surface of paw ipsilateral to the nerve ligation. The decrease of PWL and PWT indicates development of hypersensitivity to stimuli. The numbers of activated microglia and astrocytes increased after nerve injury. Values are shown as mean±SE. *$p<0.05$ vs. control (p0).

To evaluate the effect of minocycline, we compared behavioral changes and activation of microglia and astrocytes at post-ligation day 1, 4, 7, and 10. Minocycline administration was initiated 1 day before surgery and maintained till post-surgery day 9. In our preliminary study, minocycline treatment was found to attenuate thermal hypersensitivity, mechanical allodynia and activation of microglia at day 4, 7 and 10 (data not shown): Effect of minocycline was most prominent at day 10. Therefore, we decided to use the data of day 10 in the following studies. As shown in figure 2, minocycline attenuated the development of mechanical allodynia (Fig. 2B) and thermal hyperalgesia (Fig. 2A) in nerve ligated rats, and also decreased the number of activated microglia (Fig. 2C and 3C). In contrast to microglia, astrocytic activation was not significantly affected by minocycline (Fig. 3F). Since initiation time of minocycline treatment seemed to be important in our preliminary studies, we tried to gradually delay the initiation of minocycline treatment to find effective therapeutic time. The effect of minocycline was robust enough to block the hypersensitivity to thermal (Fig. 4A) and mechanical stimuli (Fig. 4B) even when drug administration was started as late as 3 days following ligation. However, the inhibitory effect of minocycline became less if the treatment was initiated at 5 days after the ligation (Fig. 4A and B). Attenuation of microglial activation by minocycline was also observed, and effective initiation time of minocycline treatment was same as shown in the behavior study (Fig. 4C).

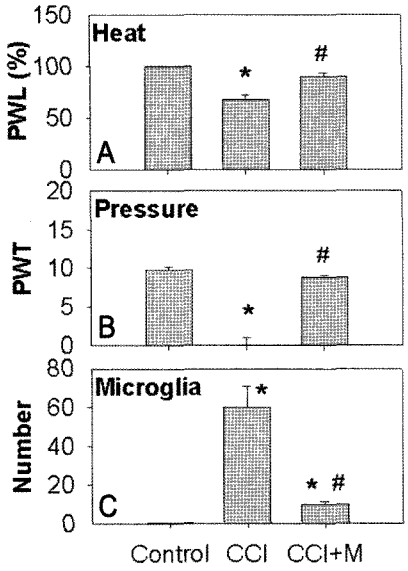

Fig. 2. Effects of minocycline on behavioral responses to thermal (A) or mechanical stimuli (B) and microglial activation (C) following sciatic nerve ligation. Minocycline (50 mg/kg, oral) was daily administered to the rats from 1 day before surgery to the last day of experiment (CCI+M). Behavioral testing was performed 10 days after surgery. Minocycline treatment significantly attenuated the behavioral changes and microglial activation induced by nerve ligation. Values are shown as mean±SE. *$p<0.05$ vs. Control; # $p<0.05$ vs. sciatic nerve ligation group (CCI).

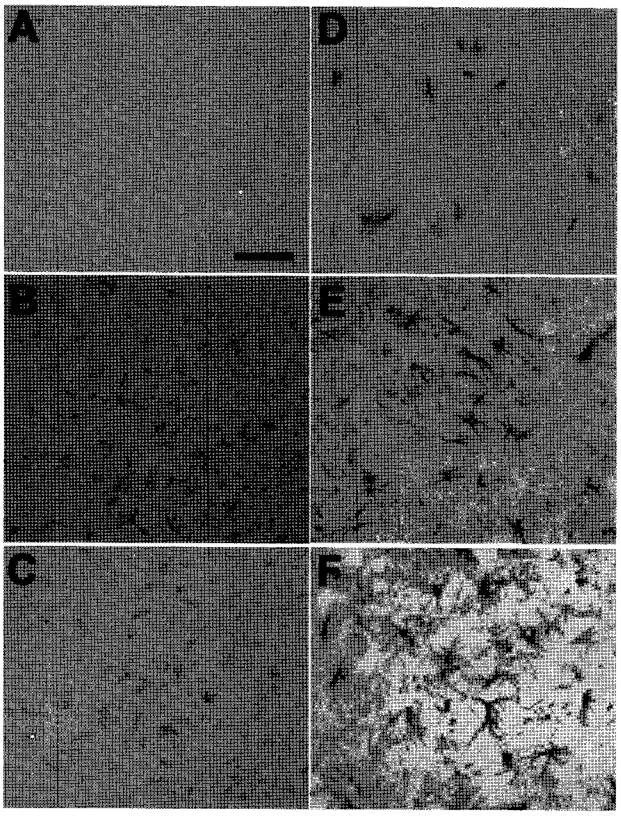

Fig. 3. Effect of minocycline on activation of microglia and astrocytes was observed 10 days after sciatic nerve ligation. Dorsal horn area of L5 spinal cord ipsilateral to the ligated nerve was observed. Before sciatic nerve ligation, there are few activated microglia (A) and small number of astrocytes (D). On 10 days after surgery, both microglial (B) and astrocytic (E) activation are prominent. Minocycline treatment attenuated the microglial (C) activation, but had no effect on astrocytes (F). Scale bar=40 μm.

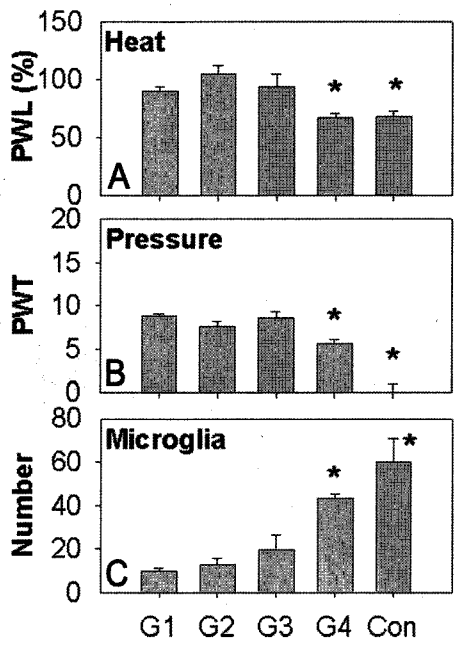

Fig. 4. Effective therapeutic time of minocycline was evaluated. Behavioral responses to thermal (A) and mechanical stimuli (B) and number of microglia (C) were evaluated 10 days after sciatic nerve ligation. Minocycline treatment was initiated from 1 day before surgery (G1) and 1 (G2), 3 (G3) and 5 days (G4) after nerve injury and given daily until the last day of experiment. The effect of minocycline is significant, even when treatment was initiated as late as 3 days after nerve ligation. The responses of sciatic nerve ligated animals without minocycline treatment (Con) were also demonstrated for the comparison of the minocycline effect. Values are shown as mean±SE. *$p<0.05$ vs. G1.

NF-kappaB activation

To study the involvement of NF-kappaB in the present model, we applied immunohistochemistry. In the normal spinal cord, NF-kappaB staining was shown mainly in the cytoplasm of large cells in the ventral spinal cord and there were few cells in the dorsal horn area (Fig. 5A). Following sciatic nerve ligation, NF-kappaB immunoreactive cells appeared in the dorsal horn area as early as 1 day following the ligation, and these cells had different sizes and shapes. The number of NF-kappaB positive cells increased in parallel with the increasing number of activated microglia in the ipsilateral dorsal horn. In the contralateral side, we could detect NF-kappaB positive cells, but the number was minimal. When compared the number of NF-kappaB immunoreactive cells at 10 days following the surgery, minocycline treatment decreased the cell numbers, compared to non-treated groups (Fig. 5B and C).

To differentiate the types of NF-kappaB immunoreactive cells, we performed double labeling of NF-kappaB and cell type markers for neuron (MAP-2), astrocyte (GFAP) and microglia (ED1). Cell type markers (brown) were visualized with diaminobenzidine (DAB) and NF-kappaB (gray black) with DAB and Nickel. In neuronal cells, NF-kappaB was present mostly in cytoplasm (Fig. 5D). In addition to cytoplasm, nuclear staining of NF-kappaB was observed in some astrocytes and microglia (Fig. 5E and F).

DISCUSSION

Of many clinical pain syndromes, painful sensations are greatly amplified, so that normally innocuous sensations, such as light touch or warmth, are perceived as pain. Presently available drugs are ineffective in controlling such pain in most patients and abolish the pain in only few cases. This failure is partly due to the fact that these drugs were developed to target neurons that transmit nociceptive information. Glial cells have recently been recognized as powerful modulators of nociception, and could hold a key to the control of pain and present a new target for drug discovery (Watkins & Maier, 2003). In this study, we evaluated the effect of minocycline, which is an antibiotic drug with function as a selective microglial activation inhibitor in various fields of nervous system disorders (Amin et al, 1996; Yrjanheikki et al, 1998; Du et al, 2001; Tikka & Koistinaho, 2001; Tikka et al, 2001a, b; Wu et al, 2002; Zhang et al, 2003).

The sciatic nerve ligated rats exhibited marked and persistent hypersensitivity to thermal and mechanical

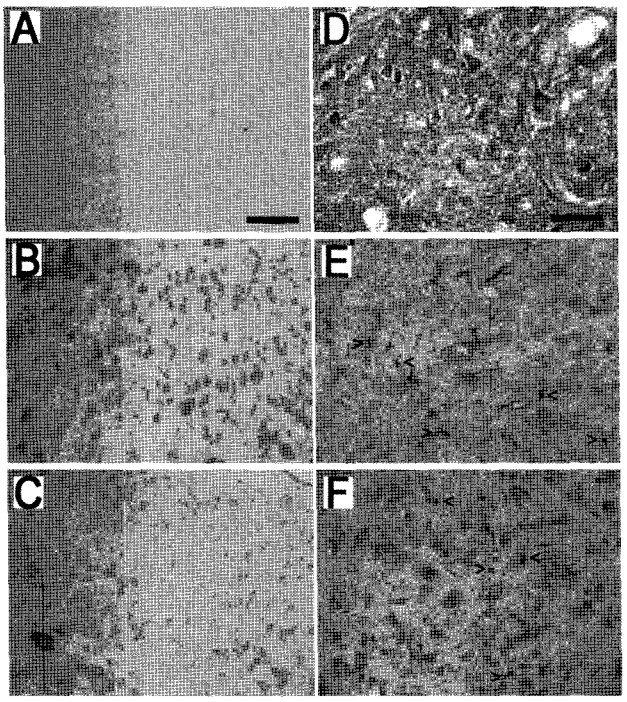

Fig. 5. NF-kappaB immunohistochemistry. Effect of minocycline on NF-kappaB was observed 10 days after nerve ligation. Dorsal horn area of L5 spinal cord ipsilateral to the ligated nerve was observed. Before sciatic nerve ligation, there are few NF-kappaB reactive cells (A). At 10 days after surgery, NF-kappaB positive cells appeared in large numbers (B). Minocycline treatment reduced the number of NF-kappaB stained cells (C). Adjacent spinal sections were double-labeled with specific cell type markers (MAP-2 for neuron, D; GFAP for astrocyte, E; ED1 for microglia, F) and NF-kappaB. Cell type markers were visualized with DAB (brown) and NF-kappaB with DAB+Nickel (dark gray). Nuclear translocation of NF-kappaB was observed mainly in microglia and astrocytes (arrow heads), but hard to find in neurons. Scale bar=40 μm.

stimuli and these responses were reversed by treatment of minocycline. To confirm the glial activation in this model and to test the effect of minocycline on glial activation, we examined the changes of microglia and astrocytes in the L4 to L6 spinal cord. The number of activated microglia and astrocytes was increased from 4 days following ligation. We could speculate that this increase was related to nerve ligation, because glial activation was prominent in the ipsilateral dorsal horn area, but not in the contralateral area. Resting microglia were observed even in the normal spinal cord, when we employed isolectin B4 histochemistry, but we could not detect any activated microglia under normal condition, when we used ED1 antibody, a specific microglial activation marker. This result suggests that nerve ligation triggers resting microglia into activation process. GFAP positive astrocytes showed similar pattern as in microglia after nerve injury. According to previous studies (Tikka et al, 2001a; Tikka & Koistinaho, 2001), minocycline selectively blocks microglial activation, but the effect on astrocytes has not been evaluated in CCI model. In the present study, minocycline effectively attenuated the activation of microglia, but did not affect astrocytes. Furthermore, minocycline reversed almost completely the behavioral changes induced by nerve injury and also dramatically reduced the number of activated microglia. Even after minocycline treatment, the number and shape of astrocytes were not significantly different from non-treated group. Therefore, it is highly likely that minocycline works selectively against microglia, and that astrocytes may not be very important during the pain development in our model. But, there still remains a possibility that astrocytes may play a role, but this was not detectable with the method used. Even though it is generally accepted that GFAP is a marker for astrocytic activation, it cannot provide functional changes perfectly. The behavior changes and glial cell activation led us to propose a hypothesis that minocycline blocked hypersensitivity to stimuli, which is related to its anti-microglial action.

In clinics, physicians usually have to treat patients several days after nerve injury. Thus, it is important to know when to start the treatment to effectively relieve pain. The effect of minocycline on behavior changes was significant, even when the initiation of treatment was delayed by 3 days following injury but it was not effective, when minocycline was started after day 5. It seems that minocycline treatment is effective, if administered before the microglial activation, however, it is not very effective against the preexisting activated microglia.

To elucidate the mechanism of minocycline on microglial activation following sciatic nerve ligation, we searched for earlier reports. Action mechanisms of minocycline other than antimicrobial activity are not well characterized until now. There are some reports to describe actions of minocycline, such as involvement in the caspase-independent and -dependent mitochondrial cell death pathways (Wang et al, 2003), inhibition of gelatinase B (Nelissen et al, 2003), inhibition of p38 mitogen activate protein kinase (MAPK, Lin et al, 2001), and inhibition of inducible nitric oxide synthase (iNOS) (Sadowski & Steinmeyer, 2001). Recent studies show that inflammatory cytokines are novel pain-enhancing substances. As for the effect of proinflammatory cytokines on pain, tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 have not been implicated in normal, non-pathological pain. By contrast, however, TNF, IL-1 and IL-6 have all been implicated in creating exaggerated pain states by their actions in spinal cord. These cytokines mediate pain induced by spinal viral challenge (HIV-1 gp120), peripheral nerve inflammation, peripheral nerve trauma, subcutaneous inflammation, and spinal dynorphin. These proinflammatory cytokines can also directly induce pain following spinal administration. Therefore, spinal proinflammatory cytokines might be necessary and sufficient for exaggerated pain states of diverse etiologies (Watkins et al, 2001a, b). The transcription factor NF-kappaB is one of the key molecules in the process of microglial activation on which several signaling pathways, elicited by lipopolysaccharide or other pro-inflammatory agents, converge (O' Neill & Kaltschmidt, 1997). NF-kappaB is a key regulator of the inflammatory cascade and many inflammatory mediators such as inflammatory cytokines, adhesion molecules and iNOS have NF-kappaB binding sequences in their promoters (Han & Yenari, 2003). In unstimulated cells, NF-kappaB exists in a latent form complexed with an inhibitory protein of the I-kappaB family, of which I-kappaB alpha is one prominent member. Upon activation, I-kappaB proteins are targeted for degradation, thus allowing the translocation of NF-kappaB to the nucleus and the transcription of target genes. Therefore,

we thought that NF-kappaB could possibly be an important regulator in the microglial activation. Indeed, in the present study, we showed that the number of NF-kappaB immunoreactive cells was increased. Furthermore, we observed that NF-kappaB was translocated into the nuclei of microglia and astrocytes in the dorsal horn area ipsilateral to the sciatic nerve ligation, and that minocycline attenuated the increase of NF-kappaB positive cells. These data might provide important clue to explain the effect of minocycline in blocking microglial activation and also help understand why astrocytic activation was not affected by minocycline. Minocycline may not alter activation pathway of astrocyte itself, but interfere with NF-kappaB related pathway, thus leading to pain modulation without affecting GFAP production. One recent study by Raghavendra and colleagues (2003) demonstrated that inhibition of microglial activation by minocycline attenuated the development, but not existing hypersensitivity in a rat neuropathy model. Although the above study employed pain model and route of drug administration different from our study, the observation made are in good support of our result and hypothesis.

Our own results together with other investigators' data led us to propose some hypotheses that 1) peripheral nerve injury provokes some changes in the spinal cord, 2) these changes lead to activation of microglia, and 3) activated microglia contributes to neuropathic pain by producing inflammatory substances. Because it is well known now that NF-kappaB is one of the most important transcription factors to induce inflammatory cytokines (Barnes PJ & Karin, 1997) and minocycline effectively inhibited hypersensitivity to stimuli, we suggest that minocycline's pain relieving action is partly through NF-kappaB pathway. Finally, we would also like to consider NF-kappaB as a candidate for microglial activation. It has been reported that NF-kappaB works in the downstream of Toll-like receptors (TLRs) (Lee & Lee, 2002): TLRs are involved in the recognition of various microbial-derived molecules, including lipopolysaccharide, lipoteichoic acid, and peptidoglycan, as well as unmethylated bacterial DNA. The TLR-mediated intracellular signaling pathways converge to activate NF-kappaB, thus inducing the transcription of a series of cytokine/chemokine genes that are involved in the initiation or regulation of the inflammatory response.

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REFERENCES

Aldskogius H, Kozlova EN. Central neuron-glial and glia-glial interactions following axon injury. *Prog Neurobiol* 55: 1–26, 1998

Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, Patel IR, Abramson SB. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. *Proc Natl Acad Sci USA* 93: 14014–14019, 1996

Aronson AL. Pharmacotherapeutics of the newer tetracyclines. *J Am Vet Med Assoc* 176: 1061–1068, 1980

Baeuerle PA, Baltimore D. NF-kappa B: ten years after. *Cell* 87: 13–20, 1996

Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066–1071, 1997

Bennett GJ, Xie YK. A peripheral mononeuropathy and neuropathic pain behaviors following peripheral seen in man. *Pain* 33: 87–107, 1998

Bethea JR, Castro M, Keane RW, Lee TT, Dietrich WD, Yezierski RP. Traumatic spinal cord injury induces nuclear factor-kappaB activation. *J Neurosci* 18: 3251–3260, 1998

Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, Bian J, Guo L, Farrell LA, Hersch SM, Hobbs W, Vonsattel JP, Cha JH, Friedlander RM. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 6: 797–801, 2000

Colburn RW, DeLeo JA, Rickman AJ, Yaeger MP, Kwon P, Hickey WF. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. *J Neuroimmunol* 79: 163–175, 1997

Colburn RW, Rickman AJ, DeLeo JA. The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. *Exp Neurol* 157: 289–304, 1999

Coyle DE. Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior. *Glia* 23: 75–83, 1998

DeLeo JA, Yezierski RP. The role of neuroinflammation and neuroimmune activation in persistent pain. *Pain* 90: 1–6, 2001

Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP, Paul SM. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci USA 98: 14669–14674, 2001

Eriksson NP, Persson JK, Aldskogius H, Svensson M. A quantitative analysis of the astroglial cell reaction in primary sensory termination areas following sciatic nerve injury and treatment with nerve growth factor. *Exp Brain Res* 114: 393–404, 1997

Garrison CJ, Dougherty PM, Kajander KC, Carlton SM. Staining of glial fibrillary acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve constriction injury. *Brain Res* 565: 1–7, 1991

Gilmore SA, Sims YJ, Leiting JE. Astrocytic reactions in spinal gray matter following sciatic axotomy. *Glia* 3: 342–349, 1990

Han HS, Yenari MA. Cellular targets of brain inflammation in stroke. *Curr Opin Investig Drugs* 4: 522–529, 2003

Kaltschmidt C, Kaltschmidt B, Neumann H, Wekerle H, Baeuerle PA. Constitutive NF-kappa B activity in neurons. *Mol Cell Biol* 14: 3981–3992, 1994

Kreutzberg, GW. Microglia: a sensor for pathological events in the CNS, *Trends Neurosci* 19: 312–318, 1990

Lee SJ, Lee S. Toll-like receptors and inflammation in the CNS. *Curr Drug Targets Inflamm Allergy* 1: 181–191, 2002

Lin S, Zhang Y, Dodel R, Farlow MR, Paul SM, Du Y. Minocycline blocks nitric oxide-induced neurotoxicity by inhibition p38 MAP kinase in rat cerebellar granule neurons. *Neurosci Lett* 315: 61–64, 2001

Ma W, Bisby MA. Increased activation of nuclear factor kappa B in rat lumbar dorsal root ganglion neurons following partial sciatic nerve injuries. *Brain Res* 797: 243–254, 1998

Meller ST, Dysktra C, Grzybycki D, Murphy S, Gebhart GF. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. *Neuropharmacology* 33: 1471–1478, 1994

Moore S, Thanos S. The concept of microglia in relation to central nervous system disease and regeneration. *Prog Neurobiol* 48: 441–460, 1996

Nelissen I, Martens E, Van den Steen PE, Proost P, Ronsse I, Opdenakker G. Gelatinase B/matrix metalloproteinase-9 cleaves interferon-beta and is a target for immunotherapy. *Brain* 126: 1371–1381, 2003

O' Neill LA, Kaltschmidt C. NF-kappa B: a crucial transcription

factor for glial and neuronal cell function. *Trends Neurosci* 20: 252–258, 1997

Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *J Pharmacol Exp Ther* 306: 624–630, 2003

Sadowski T, Steinmeyer J. Minocycline inhibits the production of inducible nitric oxide synthase in articular chondrocytes. *J Rheumatol* 28: 336–340, 2001

Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage and neurological dysfunction. *Neurosurgery* 48: 1393–1399, 2001

Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation, proliferation of microglia. *J Neurosci* 21: 2580–2588, 2001a

Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 166: 7527–7533, 2001

Tikka T, Usenius T, Tenhunen M, Keinanen R, Koistinaho J. Tetracycline derivatives and ceftriaxone, a cephalosporin antibiotic protect neurons against apoptosis induced by ionizing radiation. *J Neurochem* 78: 1409–1414, 2001b

Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, Ferrante RJ, Kristal BS, Friedlander RM. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci USA* 100: 10483–10487, 2003

Watkins LR, Maier SF. GLIA: A novel drug discovery target for clinical pain. *Nat Rev Drug Discov* 973–985, 2003

Watkins LR, Martin D, Ulrich P, Tracey K, Maier SF. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. *Pain* 71: 225–235, 1997

Watkins LR, Milligan ED, Maier SF. Spinal cord glia: new players in pain. *Pain* 93: 201–205, 2001a

Watkins LR, Milligan ED, and Maier SF. Glial activation: a driving force for pathological pain. *Trends Neurosci* 24: 450–455, 2001b

Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi D, Ischiropoulos H, Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 22: 1763–1771, 2002

Ye SM, Johnson RW. Increased interleukin-6 expression by microglia from brain of aged mice. *J Neuroimmunol* 93: 139–148, 1990

Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci USA* 95: 15769–15774, 1998

Zhang SC, Goetz BD, Duncan ID. Suppression of activated microglia promotes survival and function of transplanted oligodendroglial progenitors. Glia 41: 191–198, 2003

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16: 109–110, 1983