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Involvement of spinal tyrosine kinase in inflammatory and *N*-methyl-D-aspartate-induced hyperalgesia in rats

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Abstract

Phosphorylation of a subunit of *N*-methyl-D-aspartate (NMDA) receptor by protein tyrosine kinase (PTK) Src or Trk is known to enhance its channel activity. We examined whether a spinally administered selective PTK inhibitor, lavendustin A, which has high affinity for Src and Trk tyrosine kinases, could influence the development and maintenance of inflammatory hyperalgesia or NMDA-induced hyperalgesia. Inflammation was induced by injection of a mixture of carrageenan and kaolin into the tail base of rats. In another group of rats, hyperalgesia was induced by intrathecal administration of NMDA. Intrathecal administration of lavendustin A (1.0 μg) or NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptane-5,10-iminemaleate, MK-801 (3.0 μg) before injection of a mixture of carrageenan and kaolin or after the development of inflammation inhibited carrageenan–kaolin-induced mechanical hyperalgesia. Intrathecal injection of 1.0 μg NMDA produced thermal and mechanical hyperalgesia. Co-administration of 1.0 μg lavendustin A with NMDA significantly reduced the duration of spontaneous pain behaviour and inhibited NMDA-induced hyperalgesia. Lavendustin A itself did not cause any sedation, motor impairment or analgesia. Our results suggest that inhibition of PTK could be therapeutically effective as an analgesic in some NMDA receptor-mediated hyperalgesic states.

Keywords: Inflammatory hyperalgesia; NMDA receptor; Spinal cord; Tyrosine kinase inhibitor; Antinociception

1. Introduction

N-methyl-D-aspartate (NMDA) receptors, activated by L-glutamate, are involved in nociceptive transmission and processing within the spinal cord (Cahusac et al., 1984; Dickenson and Aydar, 1991; Woolf and Thompson, 1991). Anatomical and immunohistochemical localization of glutamate, the endogenous ligand for the NMDA receptor, to the dorsal horn of the spinal cord (Aanonsen et al., 1990) supports the role of NMDA in nociceptive processing. NMDA receptors are also involved in central sensitisation, which plays an important role in chronic pain states such as postoperative pain, arthritis and neuropathic pain. Clinically, epidural administration of ketamine, an NMDA receptor antagonist, is reported to be effective in relieving postoperative pain (Himmelseher et al., 2001; Subramaniam et al., 2001). Spinal administration of ketamine is also used in alleviating cancer pain (Kathirvel et al., 2000; Yang et al., 1996a,b). However, the action of ketamine as an intravenous anaesthetic agent limits its clinical applicability to chronic pain. Thus, there is a need to design new innovative agents targeted at the NMDA receptor.

Protein tyrosine kinases (PTKs) are widely distributed throughout the central nervous system (CNS) (Hirano et al., 1988; Wagner et al., 1991) and are thought to play an important role in signalling pathways for many extracellular molecules such as growth factors, cytokines and neurotransmitters.

Recent studies indicate that products of the immediate early genes (proto-oncogenes) Src or Fyn, both of which are known to exhibit tyrosine kinase activity, regulate the activity of the NMDA receptor, although the precise mechanisms of the regulation are not known. Wang and Salter (1994) reported that NMDA receptor-mediated whole cell currents and intracellular Ca^{2+} responses in spinal dorsal horn neurons were depressed by tyrosine kinase inhibitors, lavendustin A and genistein, and enhanced by tyrosine kinase $pp60^{c\text{-}src}$. Furthermore, Yu et al (1997) reported that the NMDA channel was regulated by channel-associated

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tyrosine kinase Src in mammalian central neurons. A recent report demonstrated that Fyn, which is a Src family protein tyrosine kinase, also influenced the activity of the NMDA receptor (Tezuka et al., 1999).

However, their role in modulation of synaptic function in the spinal cord has not been well elucidated. Recent studies reported for the first time that spinal administration of high selective Src family tyrosine kinase inhibitor, PP2, blocked complete Freund's adjuvant (CFA)-induced mechanical hyperalgesia, but it produced only a small and transient effect (Guo et al., 2002). Thus, high selectivity is not always a requirement for therapeutic efficacy.

Another potent broad-spectrum PTK inhibitor, lavendustin A, also shows a substantially high affinity for Src tyrosine kinases ($IC_{50} = 0.5$ μM), and epidermal growth factor (EGF) (Onoda et al., 1989), and low affinity for cytokine signalling pathways, such as interleukin-1β-induced interleukin-6 production (Carlson and Aschmies, 1995). Moreover, lavendustin A is known to block the effects of Trk receptor activation (Contreras 1993; Berg et al., 1995; Frerking et al., 1998; Lei et al., 1998). Trk receptor, specifically TrkB, has been recently found to modulate neuronal excitability and contribute to central sensitisation in the spinal cord (Kerr et al., 1999; Mannion et al., 1999). In addition, TrkB is also known to enhance NMDA channel activity (Levine et al., 1998; Kerr et al., 1999). Thus, inhibition of NMDA receptor activity in the spinal cord through PTK inhibition could modify the activity of the NMDA receptor and potently suppress hyperalgesia in those situations. However, little is known about the involvement of tyrosine kinase inhibitors in several pain models. In the present study, we examined the effect of intrathecally (i.t.)-administered lavendustin A in two models of hyperalgesia; (1) rats with secondary hyperalgesia induced by injection of carrageenan and kaolin into the base of the tail and (2) rats with hyperalgesia induced by spinal administration of NMDA.

2. Materials and methods

This study was approved by the Institutional Animal Care Committee, Dokkyo University School of Medicine. Male Sprague–Dawley rats (approximately 250–300 g) were prepared with chronic intrathecal catheters, which had been placed under isoflurane anaesthesia by inserting a PE-10 catheter through an incision made on the atlanto-occipital membrane to a position 8.5 cm caudal to the cisterna at the level of the lumbar enlargement. The catheter was exteriorised at the top of the skull and sealed with a piece of steel wire and the wound closed with 3–0 silk sutures. All surgical procedures were performed under sterile conditions. Intrathecal injection studies were carried out 4–6 days after surgical preparation. Rats showing neurological deficits after the catheter implantation were euthanised.

Carrageenan (Sigma, St. Louis, MO) and kaolin (Sigma), both at 4% concentrations, were mixed and a total volume of 0.4 ml was injected into the coccygeal intervertebral space at the base of the tail. Four hours after the injection, hot plate and paw pressure tests were performed every 30 min until 360 min after the injection to assess the thermal and mechanical nociceptive thresholds, respectively. For the hot plate test, the rats were tested on a 52.5 °C hot plate (Hot Plate Analgesy-Meter MK-350B, Muromachi Kikai, Tokyo, Japan). The response latency to either a hind paw lick or to a jump was recorded. When the animal did not respond within 60 s, it was removed and that score was awarded. For the paw pressure test, the hindpaw was exposed to mechanical pressure ranging from 0 to 400 g exerted on the dorsum of the paw by using a small plastic probe, and the withdrawal threshold to paw pressure was measured (Pressure Analgesy-Meter [Randall Selitto Test] MK-300, Muromachi Kikai). The baseline latency was determined twice before injection of the carrageenan–kaolin.

In a subgroup of the carrageenan–kaolin mixture injected rats, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-iminemaleate, MK-801 (3.0 μg), a noncompetitive antagonist for NMDA receptor, was intrathecally injected either 10 min before (pre) or 230 min after (post) the carrageenan–kaolin injection. The dose of MK-801 used in our study (3.0 μg) does not cause motor deficits as reported in previous studies (Ren et al., 1992; Chaplan et al., 1997). In another group of the carrageenan–kaolin-injected rats, the PTK inhibitor lavendustin A (Sigma) dissolved in 20% cyclodextrin (Sigma) solution was administered intrathecally either 10 min before (pre-treatment) or 230 min after (post-treatment) the carrageenan–kaolin injection. The dose of lavendustin A used in our study was 0.1, 0.3 or 1.0 μg. Moreover, to assess the involvement of systemic effects of lavendustin A, 1.0 μg was injected intraperitoneally (i.p.) in the same time scale. As a control, 0.4 ml saline was injected into the tail instead of a mixture of carrageenan and kaolin, or appropriate vehicle was injected intrathecally instead of lavendustin A or MK-801, respectively. Each rat was used for only one experiment prior to euthanasia with an intraperitoneal injection of a large dose of thiamylal. The acute effect of intrathecal lavendustin A 1.0 μg in noninflamed rats was also examined.

In separate series of experiments, a hyperalgesic state was induced by intrathecal injection of 1.0 μg NMDA (Sigma). NMDA was dissolved in normal saline and administered intrathecally at a volume of 10 μl. The dose of intrathecal NMDA was determined in preliminary studies; doses of NMDA larger than 3.0 μg caused severe agitation behaviour, which was indistinguishable from generalized convulsion and difficult to manipulate during application of nociceptive tests. Thus, we used 1.0 μg of NMDA in this study to produce hyperalgesia and allodynia. In these rats, the duration of spontaneous pain behaviour was measured, which represented the time between the appearance and disappearance of any of the pain-related behaviours, such as

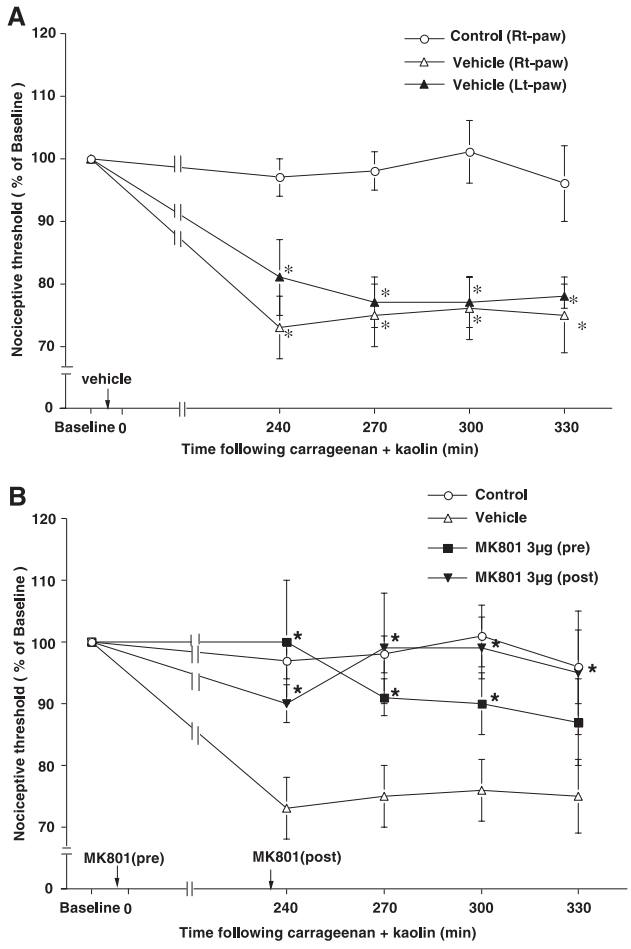

Fig. 1. Time course of mechanical nociceptive thresholds measured by paw pressure tests. Each data point represents mean ± S.E.M. of percent changes from baseline value of six to eight rats. Raw data in each group varied from 156 to 187 g and there were no significant differences among groups. Injection of a mixture of carrageenan and kaolin into the base of the tail caused significant reduction in mechanical nociceptive threshold on both the right and left paw at 240 min after the carrageenan–kaolin injection (*$P < 0.05$) (A). Carrageenan–kaolin-induced mechanical hyperalgesia was significantly blocked by both pre- and post-treatment i.t. MK-801 3.0 μg (B). Time (0) on the abscissa represents the injection of carrageenan–kaolin into the base of the tail. *$P < 0.05$, between vehicle and MK-801 groups.

vocalization, scratching, biting and licking of the flanks. After two baseline measurements, NMDA was injected intrathecally and the hot plate and paw pressure tests were performed at 15, 30, 60 and 90 min after the injection. In a separate group, PTK inhibitor, lavendustin A (Sigma) at a dose of 0.1 or 1.0 μg was co-administered with 1.0 μg NMDA. Control groups received equal volumes of the appropriate vehicle. All intrathecal drugs were injected at a final volume of 10 μl followed by an additional saline injection of 10 μl.

The behavioural changes or motor deficits induced by i.t.-administered MK-801 or lavendustin A were examined using an accelerating rotarod (MK-660B, Muromachi Kikai). All rats were given an initial three training trials to

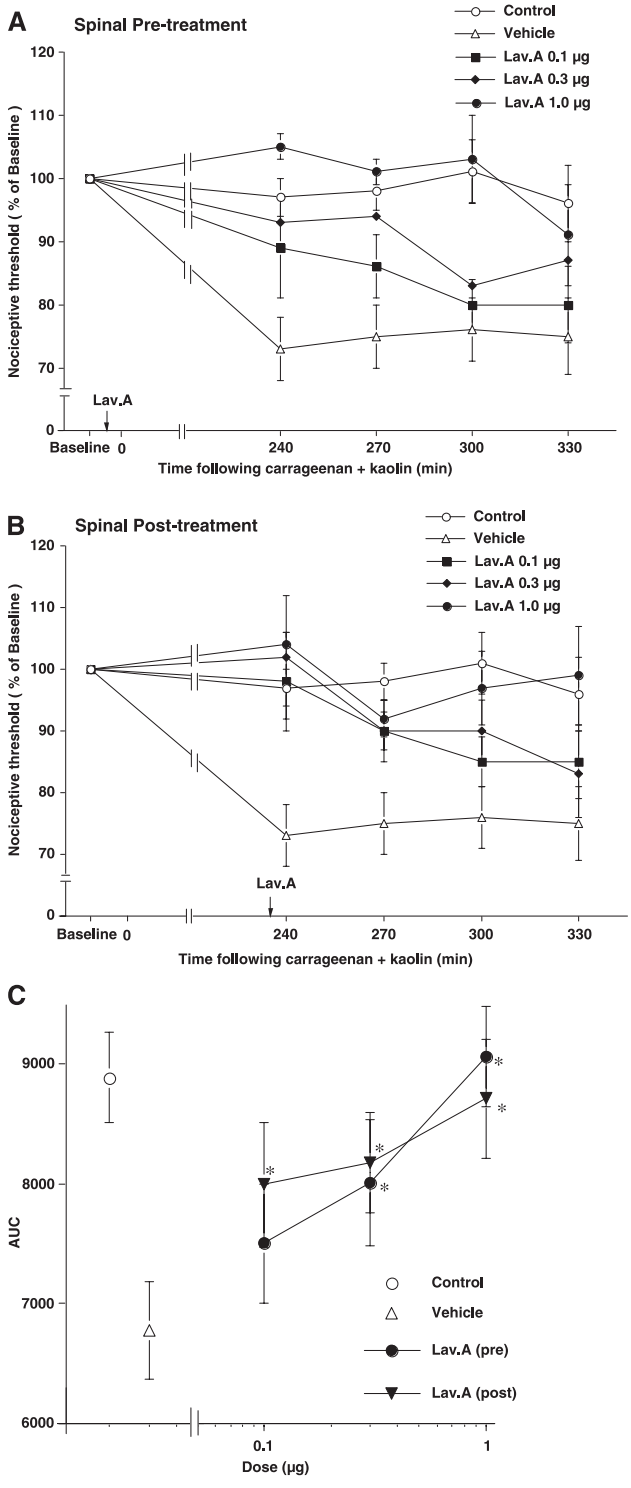

Fig. 2. Effects of i.t. lavendustin A (Lav.A) administered 10 min before (pre) (A) or 230 min after the carrageenan–kaolin (post) (B). Data of vehicle and control are identical to those in Fig. 1. Log dose–response analysis of the effect of lavendustin A (pre) or (post) was performed by calculating AUC from 240 through 330 min after the injection of the carrageenan–kaolin using the data presented in A and B to clarify the anti-hyperalgesic features of lavendustin A (C). *$P < 0.05$, compared with the vehicle group. Each data point is the mean ± S.E.M. value of six to eight rats.

maintain posture on a rotarod (diameter = 90 mm), which accelerated initially at a rate of 4 rpm, and then accelerated logarithmically to a maximum speed of 20 rpm over a period of approximately 2.5 min. When the rat fell off the rod, it tripped a timer attached to the floor of each compartment. This trial was conducted 1 h after i.t. administration of MK-801 (3.0 μg) or lavendustin A (1.0 μg).

More than six rats were assigned to each experimental group. Based on the raw data of paw pressure thresholds in grams and hot plate latencies in seconds before injection of the carrageenan–kaolin (baseline) or NMDA, the percent changes from the baseline threshold in each rat were calculated and used for statistical analyses. To analyse the effects of lavendustin A on the mechanical threshold, the area under the curve (AUC) from 240 to 360 min was calculated by the use of trapezoidal rule.

Data were analysed by the Student's *t*-test only when the mean values of two groups were compared; otherwise, one-way analysis of variance (ANOVA) was carried out with Fisher's protected least significant difference for multiple comparison. $P < 0.05$ was considered statistically significant.

3. Results

The mean baseline pressure threshold and hot plate latency in each group varied from 156 to 187 g and from 14 to 27 s, respectively, and there were no statistical differences among the groups (ANOVA).

3.1. Carrageenan–kaolin-induced inflammation

Injection of a mixture of carrageenan and kaolin into the tail produced no detectable pain behaviour, but significant mechanical hyperalgesia on both the right and left paws compared with the control group (Fig. 1A), which commenced approximately 4 h after the injection and was maintained throughout 6 h. Reliable thermal hyperalgesia was observed in the hot plate test, but was not significantly different from the control group (data not shown). Rats injected with saline into the tail exhibited no significant changes in nociceptive thresholds.

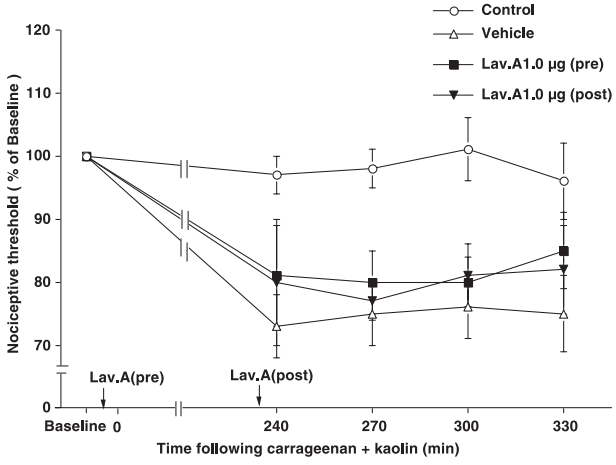

Fig. 3. Effects of systemic treatment of lavendustin A administered 10 min before (pre) or 230 min after (post) injection of the carrageenan–kaolin. Each data point is the mean ± S.E.M. value of six to eight rats.

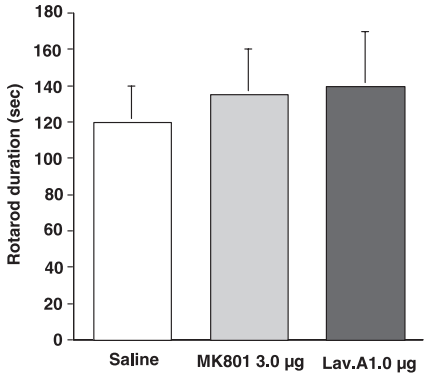

Fig. 4. Effects of MK801 or lavendustin A i.t. on motor performance in rotarod test. No motor impairment was observed in both groups. Each bar represents mean ± S.E.M. value of six to eight rats.

Pre-treatment with intrathecal MK-801 inhibited the development of mechanical hyperalgesia and the inhibitory effect was significant (Fig. 1B). Furthermore, administration of MK-801 after the development of inflammation also inhibited hyperalgesia to a level similar to that seen when the agent was injected before the carrageenan–kaolin injection (Fig. 1B). Pre-treatment with i.t. lavendustin A 1.0 μg significantly suppressed the development of mechanical hyperalgesia, which lasted more than 330 min. Pre-treatment with i.t. lavendustin A (0.3 and 0.1 μg) significantly suppressed the development of mechanical hyperalgesia during the initial 240 min after the carrageenan–kaolin injection, however, at 300 and 330 min, the effects were not significant (Fig. 2A). Post-treatment with 1.0 μg lavendustin A i.t., administered 230 min after the development of inflammation, immediately blocked the carrageenan–

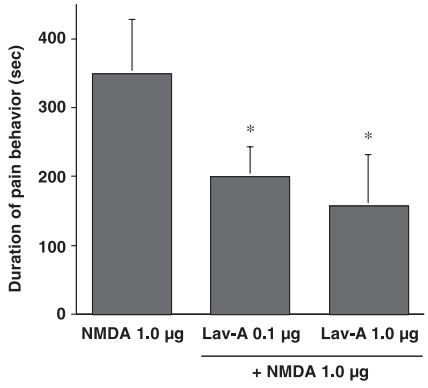

Fig. 5. Duration of pain behaviours (see Materials and methods) observed after intrathecal injection of 1.0 μg NMDA alone or with coadministration of 0.1 μg or 1.0 μg lavendustin A. Each bar represents mean ± S.E.M. value of six to eight rats. *$P < 0.05$, compared with NMDA alone.

kaolin-induced mechanical hyperalgesia and lasted more than 330 min (Fig. 2B) and lower doses of lavendustin A inhibited the maintenance of hyperalgesia during the initial 270 min, followed by a gradual decrease in mechanical nociceptive threshold. Both pre- and post-treatments with lavendustin A significantly reversed mechanical hyperalgesia on the level of AUC compared with the carrageenan–kaolin group in a dose-dependent manner (Fig. 2C). In the systemic studies, pre- or post-treatments with i.p. injection of 1.0 μg lavendustin A did not block the carrageenan–kaolin-induced mechanical hyperalgesia (Fig. 3).

I.t. injection of 1.0 μg lavendustin A in rats free of tail inflammation did not change the nociceptive threshold within 2 h after injection (Student's *t*-test; data not shown). No apparent behavioural changes or motor impairment were observed as revealed by rotarod test even at a highest dose of the i.t.-administered drugs used in the present study (Fig. 4). Sedation was not observed in the high-dose groups.

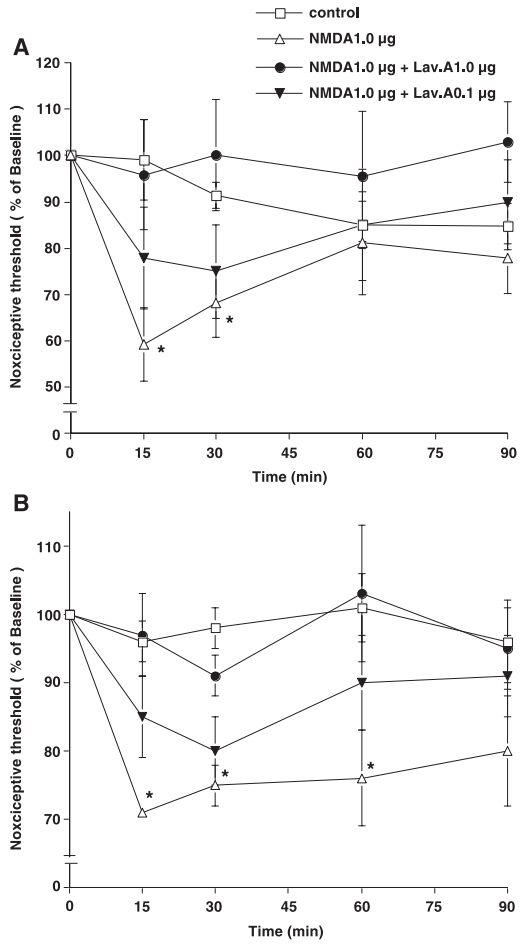

Fig. 6. Effects of intrathecal coadministration of 1.0 μg lavendustin A and NMDA on the hot plate and paw pressure tests. Intrathecal administration of 1.0 μg NMDA alone induced significant thermal (A) and mechanical hyperalgesia (B) compared with the control group (*$P<0.05$). NMDA-induced hyperalgesia was blocked by lavendustin A1.0 μg. Each data point represents the mean ± S.E.M. value of six to eight rats.

3.2. NMDA-induced hyperalgesia

Intrathecal injection of 1.0 μg NMDA produced pain-related behaviours such as spontaneous vocalization, scratching, biting and licking of the flanks, beginning within 15 s of the injection and lasting several minutes. Co-administration of lavendustin A (1.0 or 0.1 μg) did not influence the time of onset, but significantly reduced the time until all the spontaneous pain behaviours disappeared (Fig. 5). Intrathecal administration of 1.0 μg NMDA induced significant thermal and mechanical hyperalgesia compared with saline, which was detected for more than 30 min (Fig. 6A and B). Co-administration of 1.0 μg lavendustin A and NMDA completely blocked the hyperalgesia, whereas a smaller dose (0.1 μg) of lavendustin A failed to change the results of the hot plate and paw pressure tests (Fig. 6A and B).

4. Discussion

In the present study, inflammation was induced in the tail and mechanical hyperalgesia was observed in the paw. This remote hyperalgesia is often termed "secondary hyperalgesia". A considerable number of reports have described remote hyperalgesia caused by inflammation. For example, Yang et al. (1996a,b) demonstrated that intraarticular injection of a mixture of carrageenan and kaolin into the knee joint caused hyperalgesia in the ipsilateral paw distant from the site of injection. Coderre and Melzack (1991) reported the presence of thermal hyperalgesia on the contralateral leg to the burn injury. Tail injection of *Mycobacterium butyricum* caused hyperalgesia in the paw (Millan et al., 1987). Conversely, Urban et al. (1999) reported that injection of a mixture of carrageenan and kaolin in the leg produced hyperalgesia of the tail. There is ample evidence to show that NMDA receptor in the spinal cord is involved in the development of secondary hyperalgesia (Chen and Chen, 2000), which is represented by the expansion of the receptive field of spinal nociceptive neurons (Ren et al., 1992). Our results also supported the notion that NMDA receptor in the spinal cord is involved in secondary hyperalgesia, since in the present study, pre- and post-treatments with NMDA receptor antagonist MK-801 blocked the inflammation-induced remote hyperalgesia. MK-801 has an antinociceptive effect on inflammatory hyperalgesia when given intrathecally, but does not exhibit antinociception in animals free of inflammation (Yamamoto et al., 1993; Ren et al., 1992). These findings implicate the activity of NMDA receptors on spinal neurons in those animals with inflammatory hyperalgesia.

Recent reports have demonstrated that products of the immediate early genes, such as Src or Fyn, have tyrosine kinase activity and regulate the function of the NMDA receptor in vivo (Wang and Salter, 1994; Tezuka et al., 1999). Phosphorylation of a tyrosine residue on a subunit of the NMDA receptor by these PTKs causes conformational

changes and facilitates passage of cations through the channel. Thus, inhibition of Src or Fyn activities in the spinal cord by PTK inhibitors may lead to inhibition of spinal NMDA receptor activity. Similar to i.t. MK-801, our results showed that i.t. administration of 1.0 μg lavendustin A before injection the carrageenan–kaolin completely blocked mechanical hyperalgesia throughout the observation period, and that lower doses of lavendustin A delayed the onset of hyperalgesia. Since the effect of the PTK inhibitor observed in the present study was confined to animals with inflammation, the target of inhibition is probably the process implicated in neural changes caused by peripheral inflammation. Recently, Guo et al. (2002) reported that spinal post-treatment of PP2, a highly selective Src tyrosine kinase inhibitor compared with lavendustin A, blocked CFA-induced mechanical hyperalgesia, however, the effect was small and transient, suggesting that Src activation may play a lesser role in maintaining central hyperexcitability. In the present study, we showed that spinal post-treatment with 1.0 μg lavendustin A immediately blocked the carrageenan–kaolin-induced mechanical hyperalgesia, which lasted more than 330 min while lower doses of lavendustin A inhibited the maintenance of hyperalgesia during the initial 270 min. Thus, the potency of lavendustin A attenuated the maintenance of mechanical hyperalgesia induced by inflammation compared with PP2, suggesting the inhibition of not only spinal Src tyrosine kinase but also other tyrosine kinases.

The lack of peripheral effects of lavendustin A may exclude the involvement of dorsal root ganglia neurons, which are primary afferent neurons, as the target of intrathecal lavendustin A, although direct evidence could not be presented in the present study. Moreover, effects on supraspinal level could not be excluded in our study; the rapid onset of action (as early as 10 min in the post-treatment study) and the lack of effect of systemic treatment indicate that at least the primary site of action of intrathecal lavendustin A is not peripheral but at a spinal level. Taken together, these findings suggest that the inhibitory effects of lavendustin A in our study are due to inhibition of spinal NMDA receptor activity.

Other possible mechanisms of action of the PTK inhibitor must be considered. Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are known to influence nociceptive transmission (Kerr et al., 1999). In peripheral inflammation, production of NGF in peripheral Schwann cells or macrophages is upregulated and stimulates NGF receptors (TrkA) on the primary afferent fibres, which subsequently enhances production of substance P in dorsal root ganglia neurons and central transportation of substance P (Malcangio et al., 1997). NGF also stimulates BDNF production in TrkA-expressing dorsal root ganglia neurons, with BDNF transport centrally to the spinal cord (McMahon et al., 1997) and released in the dorsal horn afferent fibre stimulation (Lever et al., 2001). The increased release of substance P results in enhancement of neuronal sensitisation (Malcangio et al., 1997; Snider and McMahon, 1998; Di Luca et al., 2001). BDNF is known to facilitate NMDA receptor function (Levine et al., 1998; Lin et al., 1998) and enhances long-term potentiation in the hippocampus (Kang and Schuman, 1995). It has therefore been speculated that BDNF released in the dorsal horn may act on TrkB receptors in the spinal cord or on the nerve terminals of TrkB-expressing primary afferents, and facilitate neuronal plasticity in the spinal cord, thus enhancing inflammatory hyperalgesia (Kerr et al., 1999; Michael et al., 1997). Lavendustin A is also known to inhibit receptor type tyrosine kinases such as Trk receptors (Lei et al., 1998; Frerking et al., 1998; Hamakawa et al., 1999), and block the downstream effects of Trk receptor activation (Contreras 1993; Berg et al., 1995). Moreover, BDNF-induced phosphorylation of NR2A and 2B subunits of the NMDA receptor in the spinal cord is blocked by another broad range protein tyrosine kinase inhibitor, genistein, which is less potent than lavendustin A (Di Luca et al., 2001). In recent studies, intrathecal administration of PTK inhibitor, K-252a, a selective inhibitor of Trk tyrosine kinase, exhibited antinociceptive effects in animal models of inflammation (Sato et al., 2002) and neuropathic pain (Yajima et al., 2002). These effects lend support to the conclusion that the antinociceptive effect of intrathecal lavendustin A involves Trk in spinal cord neurons. Therefore, the present results and the above series of experiments suggest that the inhibition of development and maintenance of inflammatory hyperalgesia by spinal administration of lavendustin A might suppress not only spinal Src tyrosine kinase but also in part, trkB receptor, presumably inhibiting NMDA receptor activation.

Our results showed that NMDA-induced pain-related behaviours, thermal and mechanical hyperalgesia were inhibited by the administration of lavendustin A. Intrathecal administration of NMDA has been reported to induce not only acute thermal (Meller et al., 1996) but also mechanical hyperalgesia (Dolan and Nolan, 1999). Several studies have shown that NMDA-induced pain behaviour and hyperalgesia are only completely blocked by NMDA receptor antagonists, but not by non-NMDA or metabotropic receptor antagonists and is attenuated by inhibition of a variety of intracellular enzymes such as nitric oxide synthase (NOS), protein kinase C (PKC), and phospholipase A2 that likely results in a prolonged alteration in cellular sensitivity (Meller et al., 1996; Dolan and Nolan, 1999). Moreover, PKC-dependent potentiation of NMDA receptor function appears to be mediated via activation of Src signalling cascade (Lu et al., 1999). In the present study, intrathecally administered 1.0 μg lavendustin A completely blocked NMDA-produced hyperalgesia, suggesting that spinal delivery of lavendustin A directly inhibits tyrosine phosphorylation of spinal NMDA receptor via inhibition of Src or Fyn tyrosine kinases.

Under normal conditions, much of the TrkB receptor protein may not be available on the postsynaptic cell surface. Therefore, we think that lavendustin A did not inhibit

the TrkB activity but some other tyrosine kinase, such as Src or Fyn in the NMDA-induced hyperalgesia model, because i.t. NMDA may not be able to induce the expression of spinal trkB receptor within several minutes.

In conclusion, intrathecal administration of lavendustin A, a selective PTK inhibitor, attenuated secondary hyperalgesia induced by a mixture of carrageenan and kaolin injected into the base of the tail and hyperalgesia induced by spinal administration of NMDA. Although the neurotoxic effects of intrathecal PTK inhibitors in the treatment of inflammatory hyperalgesia are not clear, the observed anti-hyperalgesic effect of lavendustin A without apparent motor dysfunction or sedation appears to be beneficial features for a therapeutic strategy as an analgesic in some NMDA receptor-mediated hyperalgesic states. Further studies are necessary to investigate the involvement of specific tyrosine kinases in pain syndromes that possibly involve NMDA receptors.

Acknowledgements

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