Modulation of inflammatory pain in response to a CCR2/CCR5 antagonist in rodent model

Sir,

Chemokines are small (8 to 14 kDa) secreted chemotactic cytokines that interact with G-protein-coupled receptors with a highly conserved seven-transmembrane domain. They play essential roles in recruiting leukocytes to sites of injury, infection and inflammation. There are four groups of chemokines, which are divided according to their characteristic cysteine sequence motifs: The CXC, CC, CX3C and C families.

C-C chemokine ligand 2 (CCL2), previously referred to as monocyte chemoattractant protein-1 (MCP-1), a 78 amino acid secreted protein, belongs to the CC subfamily and controls the recruitment of monocytes, memory *T*-cells and natural killer cells to sites of inflammation through its chemokine receptor. CCL2 binds to Chemokine receptor type 2 (CCR2), the receptor for CCL2, exclusively and with a high affinity. CCL2 has been implicated as an important mediator of several diseases, including multiple sclerosis, rheumatoid arthritis, diabetes, and atherosclerosis. We previously reported that a CCR2 antagonist reduced swelling and joint destruction in rat models of rheumatoid arthritis and nearly produced a complete resolution of symptoms in a mouse model of multiple sclerosis.[1] In addition, recent reports suggest that chemokines may play roles in the experience of pain.[2-4] Indeed, Abbadie reported that CCR2-deficient mice showed a decrease in paw lifting and licking in a formalin-induced inflammation model. Furthermore, in response to nerve ligation, the persistent and marked up-regulation of CCR2 mRNA was evident in the nerve and dorsal root ganglia.[5] These results suggest that CCL2 may contribute to both inflammatory and neuropathic pain states. In this study, we investigated whether our CCR2 antagonist might exhibit an anti-nociceptive effect in animal models.

TLK48462, a novel dual antagonist of CCR2 and Chemokine receptor type 5 (CCR5) antagonist (CCR2/CCR5 antagonist), inhibited chemotaxis mediated by human CCL2 and human C-C chemokine ligand 4 (CCL4) with a similar potency. The chemotaxis of human PBMC (peripheral blood mononuclear cell) IC_{50} values for CCL2 and CCL4 were 1.2 μM and 1.8 μM, respectively. We also confirmed that TLK48462 inhibited chemotaxis mediated by human and rat CCL2 with similar potencies. The chemotaxis of rat spleen cells IC_{50} values was 0.4 μM.

We first evaluated the effect of TLK48462 using a formalin test. Animal care and experimental procedures were approved by the Animal Experimental Committee of Sanwa Kagaku Kenkyusho Co., Ltd. The formalin test is a widely used model that enables two different types of pain to be evaluated: Neurogenic pain is caused by the direct activation of nociceptive nerve terminals, while inflammatory pain is mediated by a combination of peripheral input and spinal cord sensitization. Seven week-old male mice were used for the study (n = 4-5). Formalin (25 μL, 1%) was injected into the plantar surface of the left paw. The time mice spent either licking or lifting the injected paw was recorded at 5 min intervals for 45 min. A test compound suspended in 5% (w/v) aqueous gum arabic or an equal volume of the vehicle was administered by gavage at 17 h and at 1 h before the formalin injection.

In the presently reported formalin test, the first phase (neurogenic phase) was observed 0-10 min after injection; the second phase (inflammatory phase) was then observed 15-45 min after injection [Figure 1a]. Mice treated with TLK48462 showed a significant reduction in paw licking and lifting during the first phase. Furthermore, TLK48462 also significantly reduced the pain during the second phase, compared with that in the control [Figure 1a and b]. The anti-nociceptive effect of TLK48462 was approximately equal to that of indomethacin, which was used as a positive control.

Next, we examined the effect of TLK48462 using a rat carrageenin model. The injection of carrageenin reportedly induces an inflammatory reaction that produces thermal or mechanical hyperalgesia. Ten week-old male Wistar rats were used for the model (n = 4-5). Inflammation was induced in the rats by the injection of 0.1 mL of 1% λ-carrageenin into

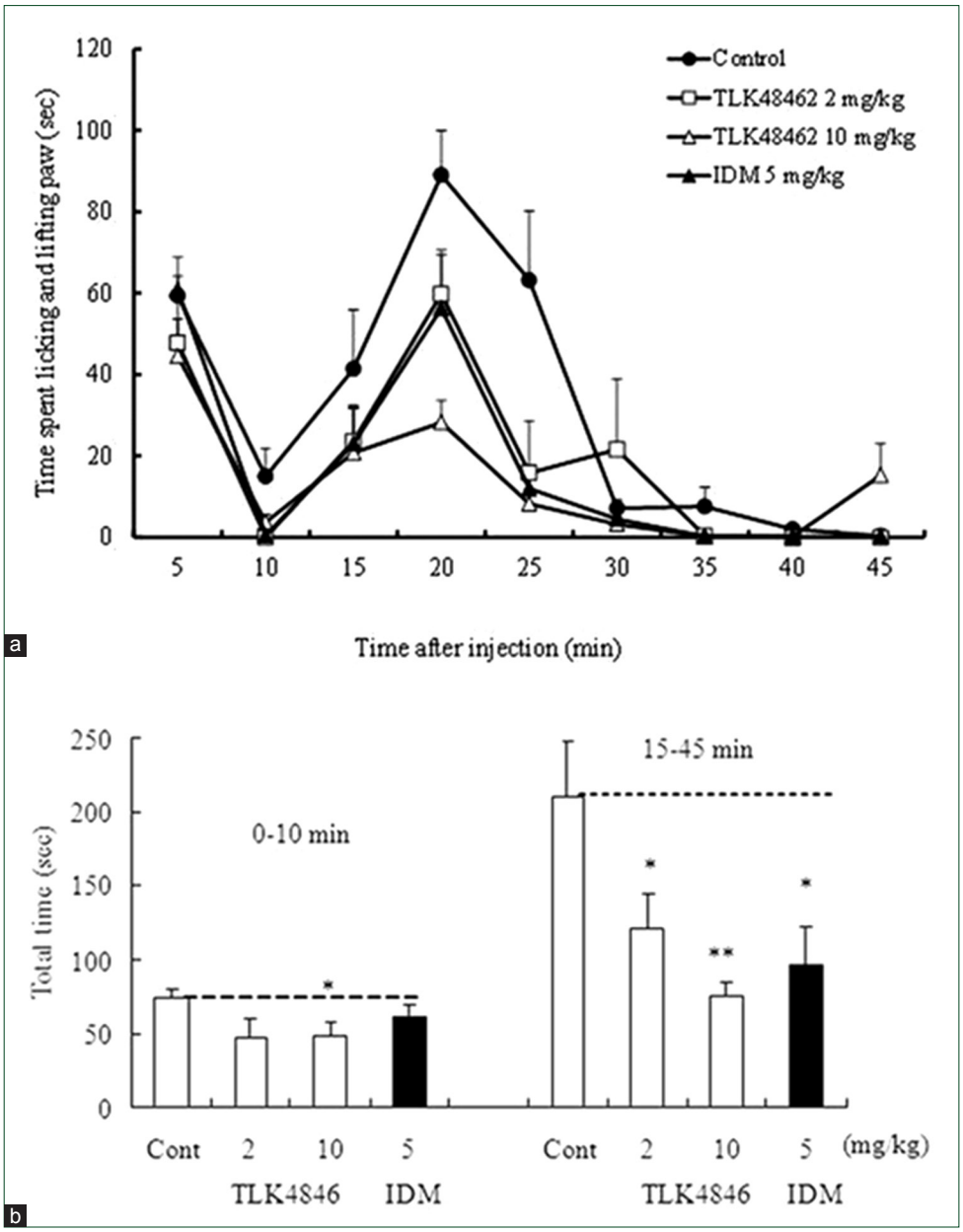

Figure 1: Effect of TLK48462 on nociceptive response to formalin injection. (a) Reduction in the duration of paw licking and lifting in response to an intraplantar injection of formalin in TLK48462-treated-mice and indomethacin (IDM)-treated mice, compared with control. (b) Area under the curve show significant decreases during phase 1 (0-10 min) and during phase 2 (15-45 min) in TKL48462-treated-mice and IDM-treated-mice, compared with control (cont). Statistical analysis was performed by Student's t-test. A P value less than 0.05 was considered as significant

the plantar surface of the right hind paw. The test compound suspended in 5% (w/v) aqueous gum arabic or an equal volume of vehicle was then administered by gavage at 17 h and at 1 h before the carrageenin injection.

The nociceptive thresholds were evaluated using the Randall and Selitto method.[6] TLK48462 reduced the carrageenin-induced hyperalgesia. The nociceptive thresholds were measured again at 4 h after the carrageenin challenge. TLK48462 also reduced the hyperalgesia at this time [Figure 2].

In this study, we confirmed the anti-nociceptive effect of TLK48462 using both formalin and carrageenin models, which we used to evaluate formalin-induced paw edema and carrageenin-induced paw edema, respectively. Interestingly, TLK48462 did not reduce formalin and carrageenin-induced paw edema (data not shown). These results suggest that TLK48462 reduces pain reactions without affecting the acute inflammatory reactions induced by formalin or carrageenin.

CONCLUSION

We revealed that TLK48462 reduced formalin-induced or

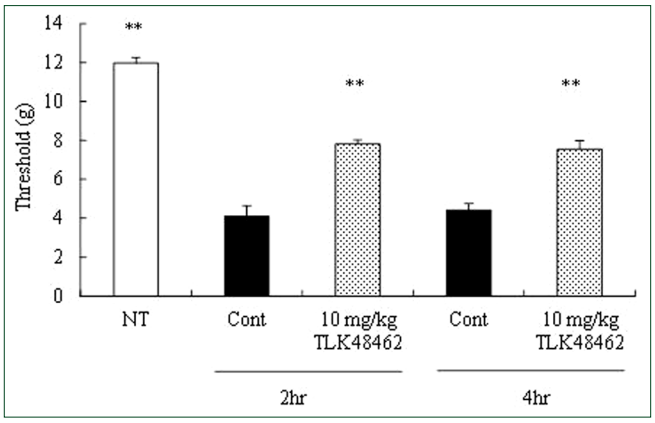

Figure 2: Effect of TLK48462 on mechanical hyperalgesia after carrageenin injection. Mechanical hyperalgesia at the carrageenin-injection site was significantly reduced in the TLK48462-treated-mice. Statistical analysis was performed by Student's *t*-test. A *P* value less than 0.05 was considered significant

carrageenin-induced pain reactions. Consequently, CCR2/CCR5 antagonists may be useful as a new therapeutic strategy for treating pain.

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Masayuki Okamoto, Takeshi Suzuki, Nobuhide Watanabe

Drug Discovery Laboratories, Sanwa Kagaku Kenkyusho Company Limited, Japan

Address for correspondence:
Masayuki Okamoto, 363, Shiosaki Hokusei-CHO, Inabe-Shi, MIE, 511-0406, Japan.
E-mail: ma_okamoto@mb6.skk-net.com

REFERENCES

1. Laborde E, Macsata RW, Meng F, Peterson BT, Robinson L, Schow SR, *et al*. Discovery, optimization, and pharmacological characterization of novel heteroaroylphenylureas antagonists of C-C chemokine ligand 2 function. J Med Chem 2011;54:1667-81.
2. Tofaris GK, Patterson PH, Jessen KR, Mirsky R. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. J Neurosci 2002;22:6696-703.
3. Milligan ED, O'Connor KA, Nguyen KT, Armstrong CB, Twining C, Gaykema RP, *et al*. Intrathecal HIV-1 envelope glycoprotein gp120 induces enhanced pain states mediated by spinal cord proinflammatory cytokines. J Neurosci 2001;21:2808-19.
4. Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. J Neurosci 2001;21:5027-35.
5. Abbadie C, Lindia JA, Cumiskey AM, Peterson LB, Mudgett JS, Bayne EK, *et al*. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc Natl Acad Sci U S A 2003;100:7947-52.
6. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther 1957;111:409-19.

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