19. Kaasa S, Mastekaasa A, Lund E. Prognostic factors for patients with inoperable non-small cell lung cancer, limited disease. The importance of patients' subjective experience of disease and psychosocial well-being. *Radiother Oncol* 1989;**15**:235–242.
20. Kukull WA, McCorkle R, Driever M. Symptom distress, psychosocial variables, and survival from lung cancer. *J Psychosoc Oncol* 1986;**4**:91–104.
21. Saito-Nakaya K, Nakaya N, Fujimori M *et al.* Marital status, social support and survival after curative resection in non-small-cell lung cancer. *Cancer Sci* 2006;**97**:206–213.
22. Nakaya N, Saito-Nakaya K, Akizuki N *et al.* Depression and survival in patients with non-small cell lung cancer after curative resection: a preliminary study. *Cancer Sci* 2006;**97**:199–205.
23. Nakaya N, Fukudo S, Akizuki N *et al.* Twenty-four-hour urinary cortisol levels before complete resection of non-small cell lung cancer and survival. *Acta Oncol* 2005;**44**:399–405.
24. Feld R, Borges M, Giner V *et al.* Prognostic factors in non-small cell lung cancer. *Lung Cancer* 1994;**11**:S19–S23.
25. Altman DG, Lyman GH. Methodological challenges in the evaluation of prognostic factors in breast cancer. *Breast Cancer Res Treat* 1998;**52**:289–303.
26. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;**12**:189–198.
27. Akechi T, Fukue-Saeki M, Kugaya A *et al.* Psychometric properties of the Japanese version of the Mental Adjustment to Cancer (MAC) scale. *Psycho-Oncology* 2000;**9**:395–401.
28. Watson M, Haviland JS, Greer S, Davidson J, Bliss JM. Influence of psychological response on survival in breast cancer: a population-based cohort study. *Lancet* 1999;**354**:1331–1336.
29. McNair D, Lorr A, Droppleman L. Manual for the Profile of Mood States. Educational and Industrial Testing Service: San Diego, 1977.
30. Yokoyama K, Araki S, Kawakami N, Tkakeshita T. [Production of the Japanese edition of profile of mood states (POMS): assessment of reliability and validity]. *Nippon Koshu Eisei Zasshi* 1990;**37**:913–918.
31. Spitzer RL, Williams JBW, Gibbon M, First MB. Structured Clinical Interview for DSM III-R. American Psychiatric Press: Washington, DC, 1990.
32. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;**67**:361–370.
33. Kugaya A, Akechi T, Okuyama T, Okamura H, Uchitomi Y. Screening for psychological distress in Japanese cancer patients. *Jpn J Clin Oncol* 1998;**28**:333–338.
34. Akechi T, Okamura H, Yamawaki S, Uchitomi Y. Predictors of patients' mental adjustment to cancer: patient characteristics and social support. *Br J Cancer* 1998;**77**:2381–2385.
35. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. *Stat Med* 2006;**25**:127–141.
36. Harrell Jr FE, Lee KL, Matchar DB, Reichert TA. Regression models for prognostic prediction: advantages, problems, and suggested solutions. *Cancer Treat Rep* 1985;**69**:1071–1077.
37. Petticrew M, Bell R, Hunter D. Influence of psychological coping on survival and recurrence in people with cancer: systematic review. *BMJ* 2002;**325**:1066.
38. Cassileth BR, Lusk EJ, Miller DS, Brown LL, Miller C. Psychosocial correlates of survival in advanced malignant disease? *N Engl J Med* 1985;**312**:1551–1555.
39. Brown KW, Levy AR, Rosberger Z, Edgar L. Psychological distress and cancer survival: a follow-up 10 years after diagnosis. *Psychosom Med* 2003;**65**: 636–643.
40. Prieto JM, Atala J, Blanch J *et al.* Role of depression as a predictor of mortality among cancer patients after stem-cell transplantation. *J Clin Oncol* 2005;**23**: 6063–6071.
41. Frasure-Smith N, Lesperance F, Talajic M. Depression following myocardial infarction. Impact on 6-month survival. *J Am Med Assoc* 1993;**270**:1819–1825.
42. Penninx BW, Beekman AT, Honig A *et al.* Depression and cardiac mortality: results from a community-based longitudinal study. *Arch Gen Psychiatry* 2001;**58**: 221–227.
43. Uchitomi Y, Yamawaki S. Truth-telling practice in cancer care in Japan. *Ann NY Acad Sci* 1997;**809**: 290–299.
44. Kugaya A, Akechi T, Okuyama T *et al.* Prevalence, predictive factors, and screening for psychologic distress in patients with newly diagnosed head and neck cancer. *Cancer* 2000;**88**:2817–2823.
45. Okamura H, Watanabe T, Narabayashi M *et al.* Psychological distress following first recurrence of disease in patients with breast cancer: prevalence and risk factors. *Breast Cancer Res Treat* 2000;**61**: 131–137.
46. Akechi T, Okuyama T, Sugawara Y *et al.* Major depression, adjustment disorders, and post-traumatic stress disorder in terminally ill cancer patients: associated and predictive factors. *J Clin Oncol* 2004;**22**: 1957–1965.
47. Block SD. Assessing and managing depression in the terminally ill patient. ACP-ASIM End-of-Life Care Consensus Panel. American College of Physicians—American Society of Internal Medicine. *Ann Intern Med* 2000;**132**:209–218.
48. Grassi L, Indelli M, Marzola M *et al.* Depressive symptoms and quality of life in home-care-assisted cancer patients. *J Pain Symptom Manage* 1996;**12**: 300–307.
49. Colleoni M, Mandala M, Peruzzotti G *et al.* Depression and degree of acceptance of adjuvant cytotoxic drugs. *Lancet* 2000;**356**:1326–1327.
50. Henriksson MM, Isometsa ET, Hietanen PS, Aro HM, Lonnqvist JK. Mental disorders in cancer suicides. *J Affect Disord* 1995;**36**:11–20.
51. Pitceathly C, Maguire P. The psychological impact of cancer on patients' partners and other key relatives: a review. *Eur J Cancer* 2003;**39**:1517–1524.
52. Prieto JM, Blanch J, Atala J *et al.* Psychiatric morbidity and impact on hospital length of stay among hematologic cancer patients receiving stem-cell transplantation. *J Clin Oncol* 2002;**20**:1907–1917.
53. Nakaya N, Goto K, Saito-Nakaya K *et al.* The lung cancer database project at the national cancer center, Japan: study design, corresponding rate and profiles of cohort. *Jpn J Clin Oncol* 2006;**36**:280–284.
54. Tammemagi CM, Neslund-Dudas C, Simoff M, Kvale P. Smoking and lung cancer survival: the role of comorbidity and treatment. *Chest* 2004;**125**: 27–37.
55. Tsoh JY, Humfleet GL, Munoz RF *et al.* Development of major depression after treatment for smoking cessation. *Am J Psychiatry* 2000;**157**:368–374.

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ROLE OF INTERLEUKIN-1β AND TUMOR NECROSIS FACTOR-α-DEPENDENT EXPRESSION OF CYCLOOXYGENASE-2 mRNA IN THERMAL HYPERALGESIA INDUCED BY CHRONIC INFLAMMATION IN MICE

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Abstract—The present study investigated whether the endogenous pro-inflammatory cytokines [interleukin (IL)-1β and tumor necrosis factor-α (TNF-α)]-dependent expression of cyclooxygenase-2 (COX-2) mRNA within the spinal cord could be involved in the development of chronic inflammatory pain-like behaviors in mice. We demonstrated that the expression of COX-2 mRNA on the ipsilateral side of the spinal cord was significantly increased 6 h and 3 days after intraplantar injection of complete Freund's adjuvant (CFA), compared with the expression in saline-treated mice. In addition, the chronic pain-like behaviors following CFA injection were markedly suppressed by repeated intrathecal (i.t.) pre-treatment with the COX-2 inhibitor etodolac, but not with the COX-1 inhibitor mofezolac. The cytosolic level of the activated form of nuclear factor-kappa B (NF-κB), which is a major contributor to the induction of COX-2, on the ipsilateral side of the mouse spinal cord was also increased compared with that in the saline-treated mice. The key finding in the present study was that a single i.t. injection with either IL-1β or TNF-α induced a marked increase in spinal COX-2 mRNA and persistent thermal hyperalgesia in mice. Furthermore, CFA-induced hypersensitivity to inflammatory pain was significantly reduced by repeated i.t. pre-injection of the recombinant Fc chimera of IL-1 receptor I or soluble TNF receptor I, which sequesters endogenous IL-1β or TNF-α, respectively. In contrast, the expression of spinal COX-2 mRNA in CFA-treated mice was similar to that in saline-treated mice at 7 days after CFA injection. The present findings strongly indicate the early intrathecal use of the COX-2 inhibitor for the relief of chronic inflammatory pain. Furthermore, together with the result in a previous study that pro-inflammatory cytokines lead to stimulation of a NF-κB-dependent transcriptional pathway, these findings suggest that a spinal cytokine/NF-κB/COX-2 pathway may play an important role in the development, but not maintenance, of chronic pain following peripheral tissue inflammation.

Key words: complete Freund's adjuvant, inflammatory pain hypersensitivity, IL-1β, TNF-α, cyclooxygenase, spinal cord.

Chronic peripheral tissue inflammation often produces a state of long-lasting pain characterized by hyperalgesia (exaggerated pain in response to painful stimuli) and allodynia (pain evoked by normally innocuous stimuli). The development of chronic pain following peripheral tissue inflammation depends both on an increase in the sensitivity of these first synapses at the site of inflammation and on an increase in the excitability of neurons in the CNS (Goppelt-Struebe and Beiche, 1997; Beiche et al., 1998). Several animal models of chronic pain following peripheral tissue inflammation have been created for to investigate the mechanisms that underlie the induction of inflammatory pain (Goppelt-Struebe and Beiche, 1997; Beiche et al., 1998). Complete Freund's adjuvant (CFA), carrageenan and formalin have been well documented to produce a state of hypersensitivity to inflammatory pain in rodents after intraplantar injection.

Early pharmacological studies on these models have demonstrated that prostaglandins (PGs) at the dorsal spinal cord as well as a peripheral site of inflammation play substantial roles in the development of hyperalgesia and allodynia (Ito et al., 2001; Zeilhofer, 2007). Prostanoids are the metabolic products of arachidonic acid by cyclooxygenase (COX). Two different isoforms of COX have been identified to date: COX-1 and COX-2 (Feng et al., 1993). Previous studies have demonstrated that COX-1 is constitutively expressed in many cell types, and COX-2 is induced at the site of inflammation, although it is also constitutively expressed in the kidney, brain and spinal cord (Vane et al., 1998; Samad et al., 2001). In response to inflammatory stimuli, once arachidonic acid is converted to PGH_2 by COX, several downstream enzymes synthesize a variety of PGs such as PGD_2, PGE_2, PGF_2, and PGI_2 (Herschman et al., 1995; Vanegas and Schaible, 2001).

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Abbreviations: CFA, complete Freund's adjuvant; COX, cyclooxygenase; EDTA, ethylenediaminetetraacetate; EGTA, ethyleneglycol-bis-β-aminoethylether-N,N,N′,N′-tetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ICR, Institute of Cancer Research; IL, interleukin; IL-1RI/Fc, recombinant human interleukin-1β receptor I chimera protein; i.t., intrathecal; NF-κB, nuclear factor-kappa B; PBS, phosphate-buffered saline; PDTC, pyrrolidinedithiocarbamate; PGs, prostaglandins; p-NF-κB, phosphorylated-nuclear factor-kappa B; RT-PCR, reverse transcription–polymerase chain reaction; SDS, sodium dodecyl sulfate; sTNFRI/Fc, recombinant human sTNF receptor I chimera protein; TBS, Tris-buffered saline; TNF-α, tumor necrosis factor-α; TTBS, Tris-buffered saline containing 0.1% Tween 20.

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Cytokine neurobiology is a relatively recent and rapidly expanding area. Most of the information about the functional roles of cytokines has been derived from studies on tissue injury and inflammation (Sweitzer et al., 1999; Hildebrand et al., 2005). A growing body of evidence suggests that the expression of pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), is markedly increased in the spinal cord of rodents with chronic pain (Inoue et al., 1999; Pahl, 1999; Sweitzer et al., 1999; Samad et al., 2001). Furthermore, it has been reported that these pro-inflammatory cytokines modulate the expression of COX-2 through nuclear factor-kappa B (NF-κB), which is implicated in gene expression, in human neuroblastoma cell and tracheal smooth muscle (Fiebich et al., 2000; Lin et al., 2004). Considering these findings, we can speculate that a spinal cytokine/NF-κB/COX-2 pathway may play an important role in the development of chronic pain following peripheral tissue inflammation.

The present study was designed to ascertain whether endogenous pro-inflammatory cytokines within the spinal cord, such as IL-1β and TNF-α, are involved in the expression of COX-2 mRNA and chronic inflammatory pain-like behaviors in mice under an inflammatory pain-like state. Additionally, we investigated the involvement of these signaling pathways within the spinal cord in the initiation or maintenance of chronic inflammatory pain following peripheral tissue inflammation.

EXPERIMENTAL PROCEDURES

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments. Animals were used only once in the present study. All behavioral experiments were conducted in a single-blind fashion to avoid the effect of subjectivity.

Animals

Male Institute of Cancer Research mice (ICR; Tokyo Laboratory Animals Science, Co., Ltd., Tokyo, Japan), weighing 22–30 g at the beginning of the experiments, were used in the present study. Animals were housed at a room temperature of 22±1 °C with a 12-h light/dark cycle (light on 8:00 am to 8:00 pm). Food and water were available *ad libitum*.

Chronic inflammatory pain model

A model of persistent inflammatory pain was produced by unilateral intraplantar injection of CFA (*Mycobacterium tuberculosis*; Sigma-Aldrich Co., MO, USA) in a volume of 50 μl into the plantar surface of the right hind paw (ipsilateral side) of mice under anesthesia with sodium pentobarbital (70 mg per kg, i.p.) (Ohsawa et al., 2000). Control mice were given saline in a volume of 50 μl into the plantar surface of the right hind paw.

Intrathecal (i.t.) injection

I.t. injection was performed as described by Hylden and Wilcox (1980) using a 25-μl Hamilton syringe with a 30 1/2-gauge needle. The needle was inserted into the intervertebral space between lumbar 5 and lumbar 6 of the spinal cord of unanesthetized mice. A reflexive flick of the tail was considered to indicate the accuracy of each injection. The injection volume for i.t. injection was 4 μl.

Measurement of thermal hyperalgesia

To assess the sensitivity to thermal stimulation, each of the hind paws of mice was tested individually using a thermal stimulus apparatus (model 33 Analgesia Meter; IITC Inc./Life Science Instruments, Woodland Hills, CA, USA). The intensity of the thermal stimulus was adjusted to achieve an average baseline paw withdrawal latency of approximately 8–10 s in naive mice. Only quick hind paw movements (with or without licking of the hind paws) away from the stimulus were considered to be a withdrawal response. Paw movements associated with locomotion or *weight-shifting* were not counted as a response. The paws were measured alternating between the left and right with an interval of more than 3 min between measurements. The latency of paw withdrawal after the thermal stimulus was determined as the average of three measurements per paw.

RNA preparation and semi-quantitative analysis by reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA obtained from the spinal cord of ICR mice was extracted using the SV Total RNA Isolation system (Promega Co., Madison, WI, USA). The lumbar spinal cord was quickly removed after mice were decapitated, and homogenized in ice-cold lysis buffer containing β-mercaptoethanol following the manufacturer's instructions. First-strand cDNA was prepared as described (Narita et al., 2001a) and the COX gene was amplified in 50 μl of a PCR solution containing $MgCl_2$, dNTP mix, and DNA polymerase with either synthesized primers (COX-1: 5′-AGG AGA TGG CTG CTG AGT TGG-3′ (sense) and 5′-AAT CTG ACT TTC TGA GTT GCC-3′ (antisense), COX-2: 5′-CTG TAT CCC GCC CTG CTG GTG-3′ (sense) and 5′-ACT TGC GTT GAT GGT GGC TGT CTT-3′ (antisense), glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5′-CCC ACG GCA AGT TCA ACG G-3′ (sense) and 5′-CTT TCC AGA GGG GCC ATC CA-3′ (antisense)). Samples were heated to 94 °C for 2 min, 55 °C for 2 min, and 72 °C for 3 min and cycled 34 times through 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min. The final incubation was at 72 °C for 7 min. The mixture was subjected to 1% agarose gel for electrophoresis with the indicated markers and primers for the internal standard (GAPDH). Each sample was applied to more than two lanes in the same gel. The agarose gel was stained with ethidium bromide and photographed with UV transillumination. The intensity of the bands was analyzed and quantified by computer-assisted densitometry using NIH Image (free download software developed by National Institutes of Health, USA). For the control, the different intensities of each band obtained from the sample from ICR mice treated with saline were analyzed, and the average intensity was calculated. Each control intensity was then compared again with the average intensity to calculate the standard error. Under these conditions, the intensities of bands for samples obtained from CFA-, IL-1β- or TNF-α-treated mice were analyzed and compared with the average intensity for ICR mice treated with saline or phosphate-buffered saline (PBS). Finally, the % of control with standard error for the each sample was quantified.

Western blotting assay

The cytosol fraction was prepared according the method described previously (Narita et al., 2001b). Briefly, the lumbar spinal cord was quickly removed after mice were decapitated, and homogenized in ice-cold buffer A containing 20 mM Tris–HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 25 μg of leupeptin per ml, 0.1 mg of aprotinin per ml and 0.32 M sucrose. The homogenate was centrifuged at 1000×g for

10 min and the supernatant was ultracentrifuged at 100,000×g for 30 min at 4 °C. The resulting supernatant was retained as the cytosolic fraction. An aliquot of tissue sample was diluted with an equal volume of electrophoresis sample buffer (Protein Gel Loading Dye-2x; AMRESCO, Solon, OH, USA) containing 2% sodium dodecyl sulfate (SDS) and 10% glycerol with 0.2 M dithiothreitol. Proteins (16 μg per lane) were applied to two lanes in duplicate, separated by size on 5–20% SDS–polyacrylamide gradient gel, and then transferred to a nitrocellulose membrane in a buffer containing 25 mM Tris and 192 mM glycine. For immunoblot detection of phosphorylated-nuclear factor-kappa B p65 (p-NF-κB), membranes were blocked in Tris-buffered saline (TBS) containing 0.3% nonfat dried milk (Nacalai Tesque, Inc., Kyoto, Japan) for 1 h at room temperature with agitation. The membrane was incubated with primary antibody diluted in TBS containing 0.3% nonfat dried milk [1:1000 p-NF-κB (Cell Signaling Technology, Inc., MA, USA)] overnight at 4 °C. The membranes were washed in TBS containing 0.1% Tween 20 (TTBS) and then incubated for 2 h at room temperature with horseradish peroxidase–conjugated goat anti-rabbit IgG (Southern Biotechnology Associates, Inc., Birmingham, AL, USA), which was diluted 1:10,000 in TBS containing 5% nonfat dried milk. After incubation, the membranes were washed in TTBS. The antigen–antibody–peroxidase complex was then finally detected by enhanced chemiluminescence (Pierce, Rockford, IL, USA) and visualized by exposure to Amersham Hyperfilm (Amersham Life Sciences, Arlington Heights, IL, USA). The membranes were washed again in TTBS and re-probed for GAPDH with primary antibody diluted in TBS containing 0.3% nonfat dried milk [1:400,000 GAPDH (Chemicon International Inc., Temecula, CA, USA)] overnight at 4 °C. Membranes were incubated with second antibody and image development was performed. Film autoradiograms were analyzed and quantified by computer-assisted densitometry using NIH Image. Each value for p-NFκB in CFA-treated mice was normalized by the respective value for GAPDH. For the control, the intensities of each band provided from the sample of saline-treated mice were analyzed, and the average intensity was calculated. Each control intensity was then again compared with the average intensity to calculate the standard error. Under these conditions, the intensity of each band for samples obtained from CFA-treated mice was analyzed and compared with the average intensity of bands from saline-treated mice. Finally, % from control with standard error for the sample of CFA-treated mice was quantified.

Drugs

The drugs used in the present study were the COX-1 inhibitor mofezolac (Mitsubishi Pharma Co., Osaka, Japan), COX-2 inhibitor etodolac (Nippon Shinyaku Co., Kyoto, Japan), the NF-κB inhibitor pyrrolidinedithiocarbamate (PDTC, Sigma-Aldrich Co., St. Louis, MO, USA), recombinant murine IL-1β (IL-1β, Pepro Tech, Inc., NJ, USA), recombinant murine TNFα/TNFSF1A (TNF-α, R&D Systems, Inc., NC, USA), recombinant human IL-1β receptor I chimera protein (IL-1RI/Fc, R&D Systems, Inc., Weaverville, NC, USA) and recombinant human sTNF receptor I chimera protein (sTNFRI/Fc, R&D Systems, Inc.). IL-1β antibody (Santa Cruz Biotechnology, CA, USA) was dissolved in 0.9% sterile physiological saline. IL-1β, TNF-α, PDTC, IL-1RI/Fc and sTNFRI/Fc were dissolved in sterile PBS. Mofezolac was dissolved in 0.9% sterile physiological saline containing 1 N Na_2CO_3. Etodolac was dissolved in 0.9% sterile physiological saline containing 0.5% carboxymethyl cellulose salt and 9% Tween 80.

Statistical analysis

All data are presented as the mean±S.E.M. The statistical significance of differences between groups was assessed with one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn multiple comparison test. The statistical significance of differences between two groups was assessed with Student's *t*-test.

RESULTS

Increase in the expression of spinal COX-2 mRNA, but not COX-1 mRNA, in the development of chronic inflammatory pain following intraplantar injection of CFA in mice

In the RT-PCR assay, the expression of COX-2 mRNA on the ipsilateral side of the spinal cord obtained from CFA-treated mice was significantly increased at both 6 h ($P<0.001$) and 3 days ($P<0.01$) after CFA injection compared with that from the saline-treated mice (Fig. 1A, B). In contrast, intraplantar injection of CFA into the mouse hind paw failed to affect mRNA levels of COX-1 on the ipsilateral spinal cord at 6 h after CFA injection compared with those on the ipsilateral side of the saline-treated mice (Fig. 1A, B). In the contralateral spinal cord obtained from CFA-treated mice, there were no changes in the expression of either COX-1 or COX-2 mRNA compared to those on the contralateral side of saline-treated mice (data not shown).

The thermal hyperalgesia observed on the ipsilateral side on day 7 after CFA injection in mice was markedly reversed by repeated i.t. injection of the preferential COX-2 inhibitor etodolac (3 and 30 nmol per mouse) 30 min before CFA treatment and once a day for seven consecutive days after CFA treatment in a dose-dependent manner (Fig. 1D). In contrast, the same treatment with the preferential COX-1 inhibitor mofezolac (10 and 30 nmol per mouse) failed to affect the decrease in the thermal threshold observed on the ipsilateral side in CFA-treated mice (Fig. 1C). Repeated i.t. injection of either mofezolac or etodolac at doses used in the present study did not affect the thermal threshold on the ipsilateral side in the saline-treated mice (Fig. 1C, D).

Changes in the latency of paw withdrawal in response to a thermal stimulus and expression of spinal COX-2 mRNA induced by a single i.t. injection of pro-inflammatory cytokines in mice

We established whether the spinal application of exogenous pro-inflammatory cytokines produces a persistent painful state in normal mice. The thermal threshold was measured at 30 min, 1, 3 and 6 h and 1, 3, 5, and 7 days after a single i.t. injection of pro-inflammatory cytokines. A single i.t. injection of either IL-1β or TNF-α caused marked thermal hyperalgesia in normal mice (Fig. 2A). The persistent painful state caused by i.t. injection of IL-1β lasted for 5 days following injection, whereas the decrease in the thermal threshold observed in TNF-α-treated mice had disappeared one day after i.t. injection of TNF-α (Fig. 2A).

Under these conditions, we next investigated the changes in the expression of COX-2 mRNA in the spinal

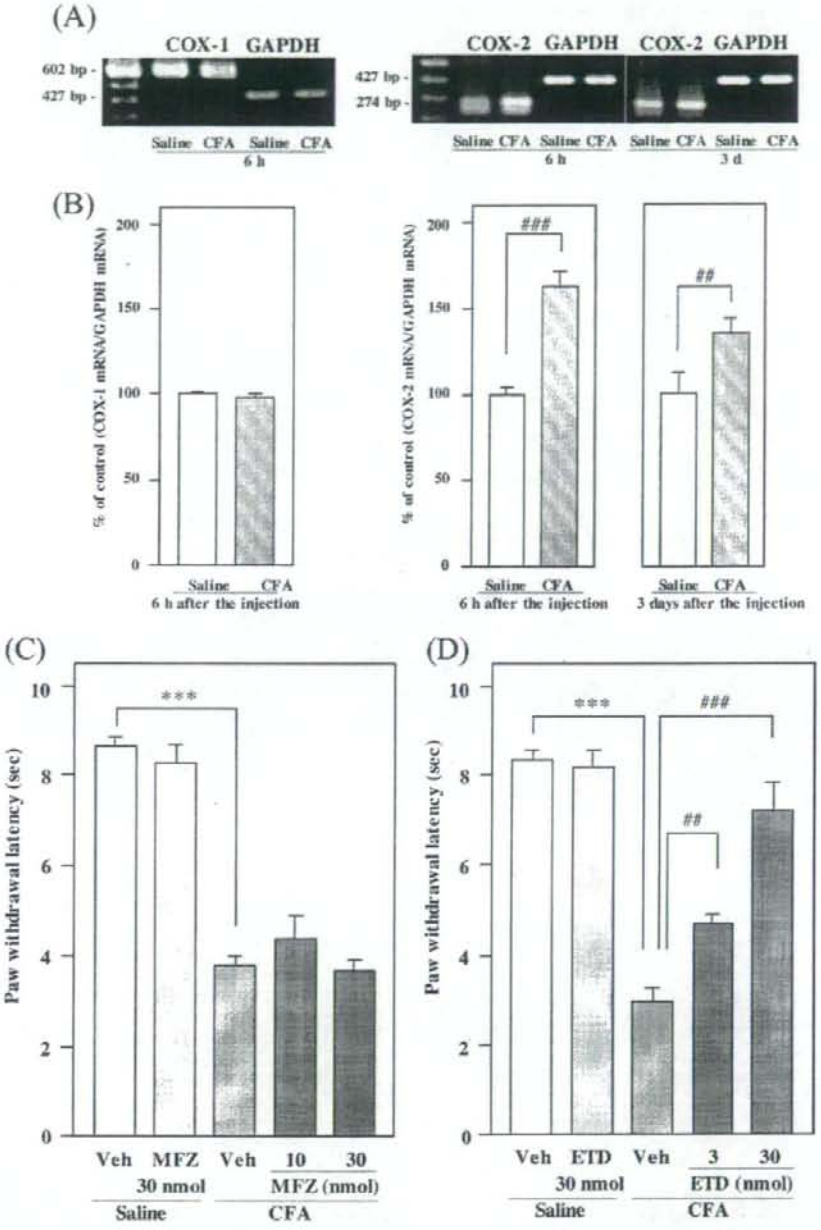

Fig. 1. (A) Representative RT-PCR for COX-1 and COX-2 mRNAs on the ipsilateral side of spinal cords obtained from the saline- or CFA-injected mice. Spinal cord samples were prepared at 6 h and 3 days after saline or CFA injection. (B) The intensity of the bands was determined semiquantitatively using NIH Image. The values for COX-1 and COX-2 mRNAs were normalized by the value for GAPDH mRNA. The value in CFA-injected mice is expressed as a percentage of the increase in saline-injected mice. Each column represents the mean±S.E.M. of three samples. $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$ vs. the saline group. (C, D) Effects of repeated i.t. pre-treatment with (C) the preferential COX-1 inhibitor mofezolac (MFZ; 10 and 30 nmol per mouse) and (D) the preferential COX-2 inhibitor etodolac (ETD; 3 and 30 nmol per mouse) on thermal hyperalgesia induced by CFA injection. The data represent paw-withdrawal latencies from a thermal stimulus on the ipsilateral side of saline- or CFA-injected mice at 7 days after injection. The i.t. injection of vehicle (Veh) or each drug was performed 30 min before CFA injection and once a day for seven consecutive days after CFA injection. On day 7, the thermal threshold was measured at 30 min after the last i.t. injection. Each column represents the mean±S.E.M. of six mice. *** $P<0.001$, Saline/Veh group vs. CFA/Veh group; $^{\#\#}$ $P<0.01$, $^{\#\#\#}$ $P<0.001$, CFA/Veh group vs. CFA/ETD group.

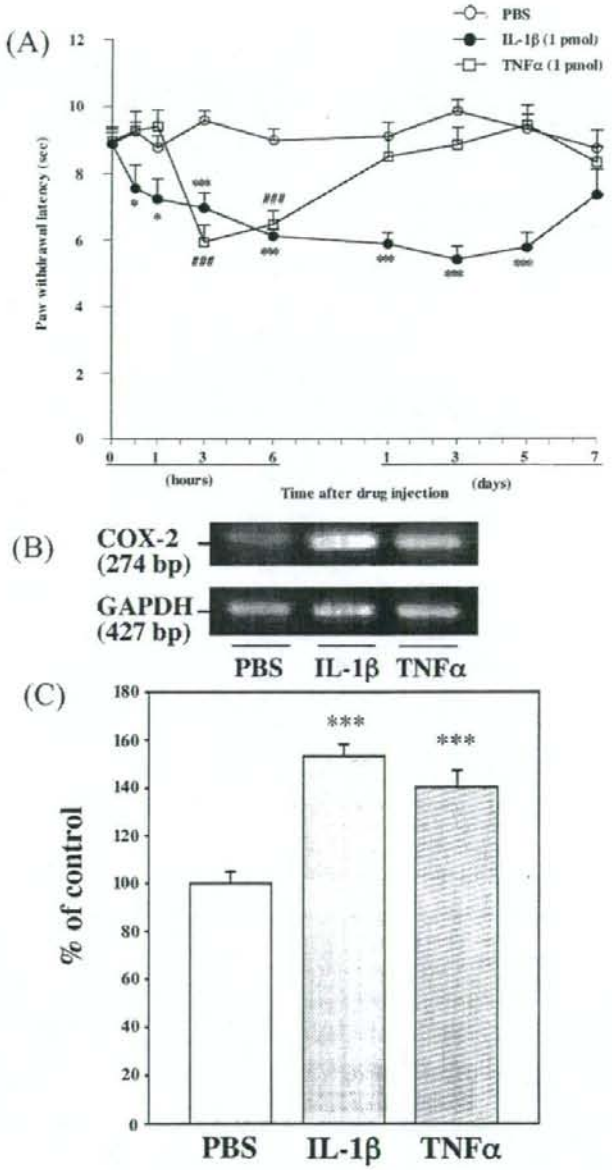

Fig. 2. (A) Time-course changes in the latency of paw withdrawal in response to a thermal stimulus induced by a single i.t. injection of pro-inflammatory cytokines in mice. The dose for i.t. injection of IL-1β and TNF-α was 1 pmol per mouse. Each point indicates the mean±S.E.M. of 5 or 6 mice. * $P<0.05$, *** $P<0.001$, PBS-injected group vs. IL-1β-injected group; and ### $P<0.001$, PBS-injected group vs. TNF-α-injected group. (B) Representative RT-PCR for mRNA of COX-2 and GAPDH, an internal standard, in the spinal cord at 3 h after the single i.t. injection of PBS, IL-1β or TNF-α in mice. (C) The intensity of the bands was determined semi-quantitatively using NIH Image. Each value for COX-2 mRNA in the pro-inflammatory cytokine-injected mice was normalized by the respective value for GAPDH mRNA. The values in pro-inflammatory cytokine-injected mice are expressed as a percentage of the increase in PBS-injected mice. Each column represents the mean±S.E.M. of nine samples. *** $P<0.001$; PBS-group vs. IL-1β or TNF-α-group.

cord of mice treated i.t. with pro-inflammatory cytokines. At 3 h after i.t. injection of either IL-1β or TNF-α, the mRNA level of spinal COX-2 was significantly increased compared with that in PBS-treated mice (Fig. 2B, C).

Effect of repeated i.t. injection of specific antibodies to IL-1β, IL-1RI/Fc, and sTNFRI/Fc on thermal hyperalgesia induced by intraplantar injection of CFA in mice

To investigate whether the release of endogenous IL-1β within the spinal cord directly contributes to the development of inflammatory pain-related hypersensitivity induced by the intraplantar injection of CFA, mice were treated i.t. with IL-1β antibody (8.0 pg per mouse) or IL-1RI/Fc (10 ng per mouse) 1 h before and once a day for seven consecutive days after CFA injection. The thermal threshold was measured 1 h after the last i.t. injection of neutralizing antibodies or recombinant receptor chimera proteins on each test day. Repeated i.t. treatment with either IL-1β antibody or IL-1RI/Fc significantly reversed the decreased thermal threshold on the ipsilateral side in CFA-treated mice (Fig. 3A and B). Treatment with IL-1β antibody had no effect on the latency of paw withdrawal after the application of a thermal stimulus on the contralateral side in the CFA-treated mice. The inhibition of persistent painful state observed in IL-1β antibody-treated mice lasted for more than 14 days following CFA treatment, even though repeated i.t. injection of IL-1β antibody was discontinued days 8–14 after CFA injection.

Similarly, repeated i.t. injection of sTNFRI/Fc (10 and 30 ng per mouse) 1 h before and once a day for seven consecutive days after CFA injection significantly inhibited thermal hyperalgesia induced by CFA treatment (Fig. 3C). The same treatment had no effect on the latency of paw withdrawal after the application of a thermal stimulus on the contralateral side in the CFA-treated mice (data not shown).

Implication of spinal NF-κB in the development of chronic inflammatory pain in mice treated with CFA

For immunoblot detection, we used polyclonal antibody that detected phosphorylated NF-κB p65 at serine 536. In the present study, the cytosolic level of p-NF-κB p65 on the ipsilateral side of the spinal cord obtained from CFA-injected mice was significantly increased ($P<0.001$) compared to that in saline-treated mice at 3 h after CFA injection (Fig. 4A, B).

We next investigated the effect of repeated i.t. injection of the NF-κB inhibitor PDTC on the persistent painful state caused by the injection of CFA. Repeated i.t. injection of vehicle or PDTC (10, 30 and 100 pmol per mouse) was performed 30 min before and once a day for seven consecutive days after saline or CFA injection. The thermal threshold was measured 30 min after the last i.t. injection of vehicle or PDTC at 7 days after saline or CFA injection. Under these conditions, the thermal hyperalgesia observed on the ipsilateral side on day 7 after CFA injection in mice was reversed by repeated i.t. injection of PDTC in a dose-dependent manner (Fig. 4C). Repeated i.t. injection of PDTC (100 pmol per mouse) did not affect the thermal threshold on the ipsilateral side in saline-treated mice (Fig. 4C).

Implication of spinal COX-2 mRNA in maintenance of the chronic inflammatory pain following the intraplantar injection of CFA in mice

Next, we investigated whether the expression of COX-2 mRNA is implicated in the maintenance of the chronic inflammatory pain induced by intraplantar injection of CFA. The expression of COX-2 mRNA on the ipsilateral side of the spinal cord obtained from CFA-treated mice was similar to that found in the saline-treated mice at 7 days after CFA injection (Fig. 5A, B), whereas the thermal hyperalgesia induced by CFA was observed on the ipsilateral side (Fig. 1). Similarly, there were differences in the expression of spinal COX-1 mRNA between CFA- and saline-treated mice (data not shown).

We next evaluated the effect of repeated i.t. post-treatment with etodolac (30 nmol/mouse) on the thermal hyperalgesia induced by CFA injection. Repeated i.t. treatment with etodolac from day 7 to day 15 after CFA injection failed to reverse the decreased thermal threshold on the ipsilateral side in CFA-treated mice (Fig. 5C).

DISCUSSION

In the present study, the expression of COX-2 mRNA on the ipsilateral side of the spinal cord obtained from CFA-treated mice was significantly increased during the developmental phase (6 h and 3 days after CFA injection) of inflammatory pain-related hypersensitivity compared with that on the ipsilateral side in saline-treated mice. In addition, the chronic pain-like behaviors following CFA injection were markedly suppressed by repeated i.t. pre-treatment with the preferential COX-2 inhibitor etodolac (30 min before CFA treatment and once a day for seven consecutive days after CFA treatment), but not the preferential COX-1 inhibitor mofezolac. Furthermore, mice that had been treated i.t. with etodolac exhibited a significant inhibition of thermal hyperalgesia induced by CFA treatment, even though the injection was interrupted on days 8–14 after CFA injection. These data can be partly supported by a previous report by Samad et al. (2001), who stated that the inflammatory pain-related hypersensitivity induced by CFA was significantly suppressed by a single i.t. injection of the COX-2 inhibitor NS398 48 h after CFA injection. Furthermore, previous studies have demonstrated that a substantial upregulation of COX-2 protein can be detected in the superficial dorsal horn of the spinal cord after the intraplantar injection of CFA (Samad et al., 2001; Yamada et al., 2006). The present findings provide further evidence that COX-2, but not COX-1, within the spinal cord plays a role in the development of chronic pain following peripheral tissue inflammation.

A growing body of evidence suggests that pro-inflammatory cytokines serve as neuromodulators and neurotransmitters in the CNS (Samad et al., 2001; Davies et al., 2006). They can be broadly classified into four major groups: IL growth factors, interferons, chemokines and TNF. Among these, cytokines, IL-1β and TNF-α are strongly implicated in chronic pain as well as immune responses at peripheral sites and the CNS (Samad et al.,

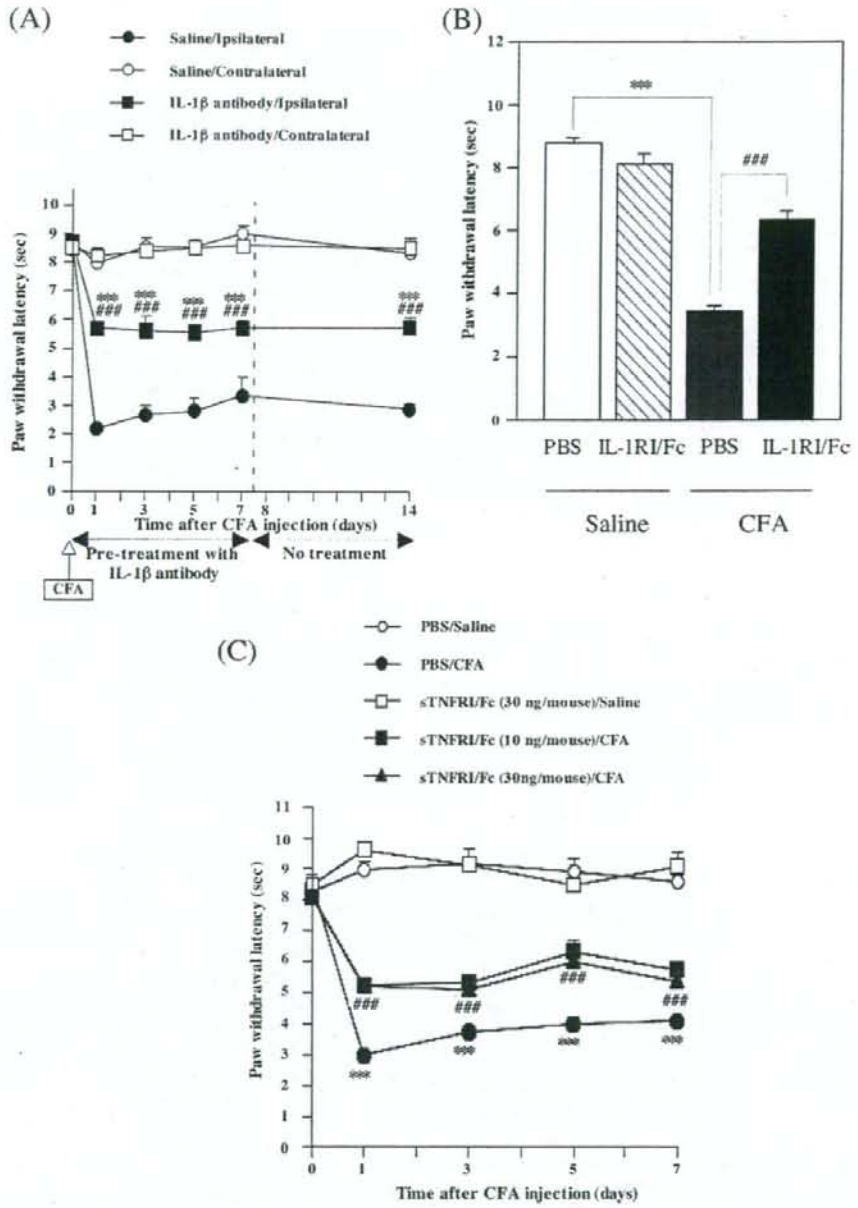

Fig. 3. (A) Effect of repeated i.t. pre-treatment with the specific IL-1β antibody on thermal hyperalgesia induced by CFA injection. The i.t. injection of saline or IL-1β antibody (8.0 pg per mouse) was performed 1 h before CFA injection and once a day for seven consecutive days after CFA injection. During days 8–14 after CFA injection, the antibody was not given to the mouse. Each point indicates the mean±S.E.M. of six mice. ### $P<0.001$, saline/ipsilateral side group vs. IL-1β antibody/ipsilateral side group; *** $P<0.001$, IL-1β antibody/ipsilateral side group vs. IL-1β antibody/contralateral side group. (B) Effect of repeated i.t. injection of IL-1RI/Fc on the latency of paw withdrawal in response to a thermal stimulus on the ipsilateral side in PBS- or CFA-injected mice. The repeated i.t. injection of PBS or IL-1RI/Fc (10 ng per mouse) was performed 1 h before and once a day for seven consecutive days after saline or CFA injection. The thermal threshold was measured 1 h after the last i.t. injection of PBS or IL-1RI/Fc at 7 days after saline or CFA injection. Each point represents the mean±S.E.M. of 4 to 6 mice. *** $P<0.001$, PBS/saline group vs. PBS/CFA group; ### $P<0.001$, PBS/CFA group vs. IL-1RI/Fc/CFA group. (C) Effect of repeated i.t. injection of sTNFRI/Fc on the latency of paw withdrawal in response to a thermal stimulus on the ipsilateral side in saline- or CFA-injected mice. The repeated i.t. injection of PBS or sTNFRI/Fc (10 or 30 ng per mouse) was performed 1 h before and once a day for seven consecutive days after saline or CFA injection. Each point represents the mean±S.E.M. of 4 to 6 mice. *** $P<0.001$, PBS/Saline group vs. PBS/CFA group; ### $P<0.001$, PBS/CFA group vs. sTNFRI/Fc (10 or 30 ng per mouse)/CFA group.

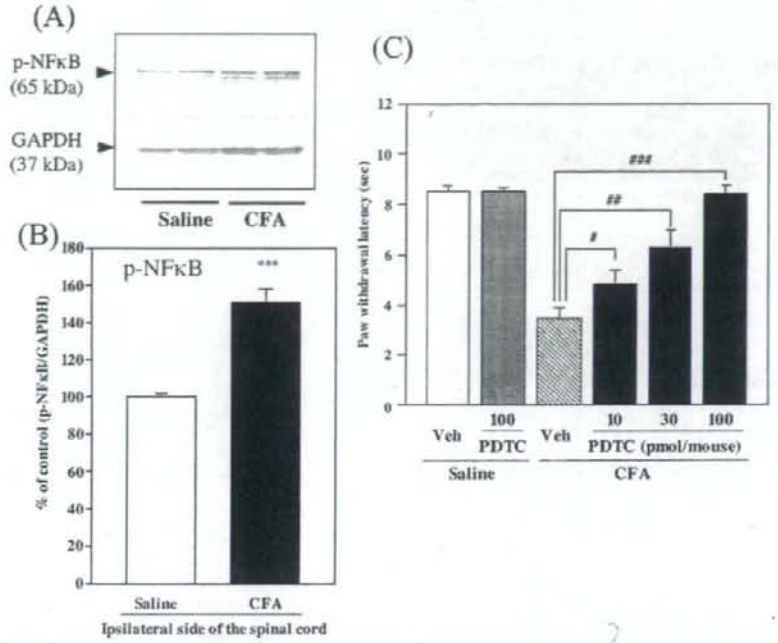

Fig. 4. (A) Representative Western blot of cytosolic p-NF-κB p65 protein and GAPDH as a control for loading. (B) Change in the cytosolic level of p-NF-κB on the ipsilateral side of the spinal cord obtained from CFA-injected mice. The cytosolic fraction was prepared at 3 h after saline or CFA injection. p-NF-κB immunoreactivity in CFA-injected mice is expressed as a percentage increase compared with saline-injected mice. Each column represents the mean±S.E.M. of eight samples. *** $P<0.001$; Saline group vs. CFA group. (C) Effect of repeated i.t. injection of the NF-κB inhibitor PDTC on the latency of paw withdrawal in response to a thermal stimulus on the ipsilateral side in saline- or CFA-injected mice. The repeated i.t. injection of vehicle (Veh) or PDTC (10, 30 and 100 pmol per mouse) was performed 30 min before and once a day for seven consecutive days after saline or CFA injection. The thermal threshold was measured 30 min after the last i.t. injection of Veh or PDTC at 7 days after saline or CFA injection. Each column represents the mean±S.E.M. of 3 to 7 mice. # $P<0.05$, ## $P<0.01$, and ### $P<0.001$; Veh/CFA group vs. PDTC/CFA group.

2001; Davies et al., 2006). It has been reported that either IL-1β or TNF-α is rapidly and transiently expressed in microglia and astrocytes in the injured mouse spinal cord, indicating that microglia and astrocytes are the major sources of pro-inflammatory cytokines in the spinal cord (Pineau and Lacroix, 2007). Several lines of evidence have suggested that these pro-inflammatory cytokines, which are released in the spinal cord in response to inflammatory stimuli, lead to the stimulation of a NF-κB-dependent transcriptional pathway (Lee et al., 2004). It is well known that NF-κB is involved in various pathological processes, particularly chronic inflammation, neurodegeneration, atherosclerosis and cancer (Pahl, 1999). In response to various inflammatory or other stress stimuli, NF-κB is activated and translocated into the nucleus from the cytosol. After binding to DNA sites of NF-κB-responsive genes, NF-κB positively regulates the transcription of numerous genes including cytokines, chemokines, adhesion factors and pro-inflammatory enzymes (nitric oxide synthase and COX-2) (DiDonato et al., 1997; Lukiw and Bazan, 1998; Ledeboer et al., 2005).

In the present study, we demonstrated that a single i.t. injection of either IL-1β or TNF-α induced a marked increase in spinal COX-2 mRNA and persistent thermal hyperalgesia in mice. In addition, CFA-induced inflammatory pain-related hypersensitivity was significantly reduced by repeated i.t. pre-injection of the IL-1β antibody, IL-1RI/Fc and sTNFRI/Fc. IL-1RI/Fc and sTNFRI/Fc are bivalent homodimers that contain the extracellular ligand-binding domain of a given IL-1 or TNF receptor, respectively, followed by the hinge and Fcγ region of human IgG1 (Sims et al., 1988; Lewis et al., 1991; Kumar et al., 1995). These antibody and receptor chimera proteins act as IL-1 or TNF receptor bodies to sequester endogenous IL-1β or TNF-α, and are highly potent and specific inhibitors for endogenous IL-1β or TNF-α, respectively. Considering these findings, the present data suggest that the IL-1β and TNF-α released within the spinal cord play a critical role in the development of chronic inflammatory pain induced by the intraplantar injection of CFA. The cytosolic level of p-NF-κB, which is an activated form of NF-κB, on the ipsilateral side of the spinal cord was markedly increased compared with that in the saline-treated mice at 3 h after CFA injection. Furthermore, the thermal hyperalgesia on day 7 after CFA injection was markedly reversed by repeated i.t. injection of the NF-κB inhibitor PDTC in mice. Taken together, these findings indicate that the IL-1β or TNF-α released within the spinal cord after the intraplantar injec-

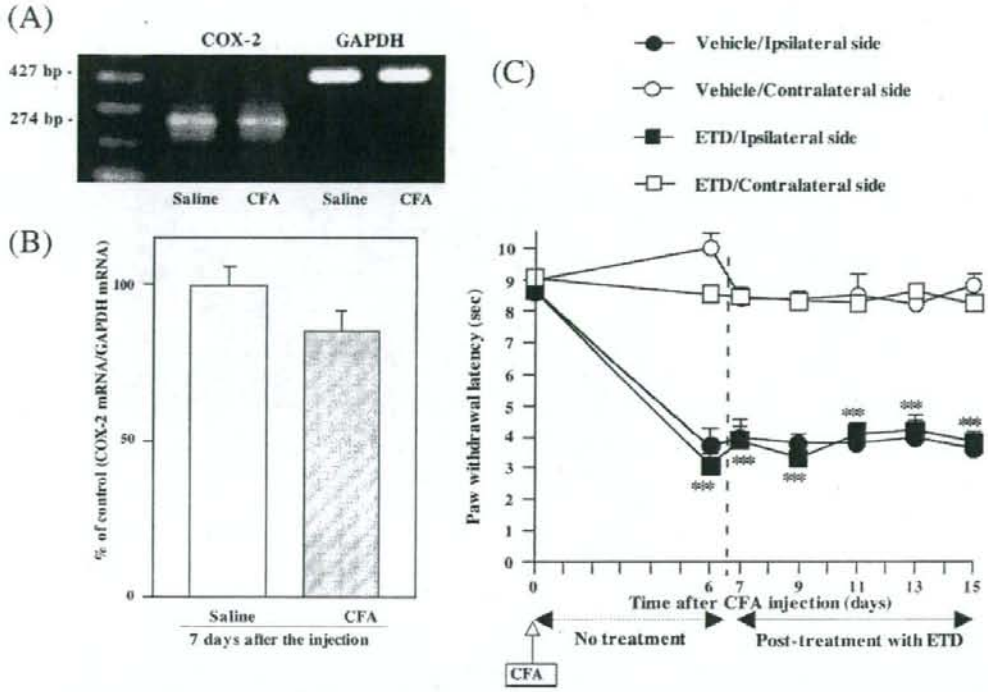

Fig. 5. (A) Representative RT-PCR for COX-2 mRNA on the ipsilateral side of spinal cords obtained from the saline- or CFA-injected mice. Spinal cord samples were prepared at 7 days after saline or CFA injection. The samples were subjected to 1% agarose gel electrophoresis with the indicated markers and primers for the internal standard GAPDH. (B) The intensity of the bands was determined semi-quantitatively using NIH Image. The value for COX-2 mRNA was normalized by the value for GAPDH mRNA. The value in CFA-injected mice is expressed as a percentage of the increase in saline-injected mice. Each column represents the mean±S.E.M. of three samples. (C) Effect of repeated i.t. post-treatment with the preferential COX-2 inhibitor etodolac (ETD; 30 nmol per mouse) on the thermal hyperalgesia induced by CFA injection. Repeated i.t. post-treatment with vehicle or ETD was performed from days 7–15 after CFA injection. During the first 6 days after CFA injection (days 0–6), mice were not treated with vehicle or ETD. Each point indicates the mean±S.E.M. of six mice. *** $P<0.001$, ETD/ipsilateral side group vs. ETD/contralateral side group.

tion of CFA may lead to the activation of NF-κB and a subsequent increase in COX-2 mRNA expression through a NF-κB-dependent transcriptional pathway. Thus, it seems likely that these signaling pathways in the spinal cord are responsible for the development of the chronic inflammatory pain-like state in CFA-treated mice.

We further evaluated the specific involvement of COX-2 within the spinal cord in the maintenance of the chronic inflammatory pain-like state in CFA-treated mice. Interestingly, there were no differences in the expression of spinal COX-2 mRNA between CFA- and saline-treated mice at 7 days after CFA injection. These findings provide evidence that the expression of COX-2 mRNA within the spinal cord is not necessarily associated with the maintenance of an inflammatory pain-like state. This notion can be further supported by the present behavioral study that repeated i.t. treatment with etodolac during days 7–15 after CFA injection failed to affect the decreased thermal threshold on the ipsilateral side of CFA-treated mice, whereas chronic pain-like behaviors following CFA injection were markedly suppressed by repeated i.t. pre-treatment with etodolac just before and seven consecutive days after CFA treatment. Thus, it seems likely that distinct mechanisms are associated with the development and maintenance of the inflammatory pain-like state following CFA treatment. Furthermore, the present findings strongly indicate the early use of the COX-2 inhibitor to relieve chronic inflammatory pain.

CONCLUSION

In conclusion, we have demonstrated that the release of pro-inflammatory cytokines, IL-1β and TNF-α, within the spinal cord after the intraplantar injection of CFA may lead to the activation of NF-κB and of COX-2 mRNA expression through a NF-κB-dependent transcriptional pathway. We propose here that these signaling pathways in the spinal cord may be responsible for the development, but not the maintenance, of a chronic inflammatory pain-like state in CFA-treated mice.

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REFERENCES

Beiche F, Brune K, Geisslinger G, Goppelt-Struebe M (1998) Expression of cyclooxygenase isoforms in the rat spinal cord and their regulation during adjuvant-induced arthritis. Inflamm Res 47: 482–487.

Davies AL, Hayes KC, Shi R (2006) Recombinant human TNFα induces concentration-dependent and reversible alterations in the electrophysiological properties of axons in mammalian spinal cord. J Neurotrauma 23:1261–1273.

DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M (1997) A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. Nature 388:548–554.

Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB, Hwang D (1993) Cloning two isoforms of rat cyclooxygenase: differential regulation of their expression. Arch Biochem Biophys 307:361–368.

Fiebich BL, Mueksch B, Boehringer M, Hull M (2000) Interleukin-1β induces cyclooxygenase-2 and prostaglandin E(2) synthesis in human neuroblastoma cells: involvement of p38 mitogen-activated protein kinase and nuclear factor-κB. J Neurochem 75:2020–2028.

Goppelt-Struebe M, Beiche F (1997) Cyclooxygenase-2 in the spinal cord: localization and regulation after a peripheral inflammatory stimulus. Adv Exp Med Biol 433:1213–1216.

Herschman HR, Xie W, Reddy S (1995) Inflammation, reproduction, cancer and all that.... The regulation and role of the inducible prostaglandin synthase. Bioessays 17:1031–1037.

Hildebrand F, Pape HC, Krettek C (2005) [The importance of cytokines in the posttraumatic inflammatory reaction.] Unfallchirurg 108: 793–794.

Hylden JL, Wilcox GL (1980) Intrathecal morphine in mice: a new technique. Eur J Pharmacol 67:313–316.

Inoue A, Ikoma K, Morioka N, Kumagai K, Hashimoto T, Hide I, Nakata Y (1999) Interleukin-1β induces substance P release from primary afferent neurons through the cyclooxygenase-2 system. J Neurochem 73:2206–2213.

Ito S, Okuda-Ashitaka E, Minami T (2001) Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res 41:299–332.

Kumar S, Minnich MD, Young PR (1995) ST2/T1 protein functionally binds to two secreted proteins from Balb/c 3T3 and human umbilical vein endothelial cells but does not bind interleukin 1. J Biol Chem 270:27905–27913.

Ledeboer A, Gamanos M, Lai W, Martin D, Maier SF, Watkins LR, Quan N (2005) Involvement of spinal cord nuclear factor kappaB activation in rat models of proinflammatory cytokine-mediated pain facilitation. Eur J Neurosci 22:1977–1986.

Lee KM, Kang BS, Lee HL, Son SJ, Hwang SH, Kim DS, Park JS, Cho HJ (2004) Spinal NF-κB activation induces COX-2 upregulation and contributes to inflammatory pain hypersensitivity. Eur J Neurosci 19:3375–3381.

Lewis M, Tartaglia LA, Lee A, Bennett GL, Rice GC, Wong GH, Chen EY, Goeddel DV (1991) Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. Proc Natl Acad Sci U S A 88: 2830–2834.

Lin CC, Sun CC, Luo SF, Tsai AC, Chien CS, Hsiao LD, Lee CW, Hsieh JT, Yang CM (2004) Induction of cyclooxygenase-2 expression in human tracheal smooth muscle cells by interleukin-1β: involvement of p42/p44 and p38 mitogen-activated protein kinases and nuclear factor-kappaB. J Biomed Sci 11:377–390.

Lukiw WJ, Bazan NG (1998) Strong nuclear factor-κB-DNA binding parallels cyclooxygenase-2 gene transcription in aging and in sporadic Alzheimer's disease superior temporal lobe neocortex. J Neurosci Res 53:583–592.

Narita M, Mizoguchi H, Suzuki T, Narita M, Dun NJ, Imai S, Yajima Y, Nagase H, Suzuki T, Tseng LF (2001a) Enhanced μ-opioid responses in the spinal cord of mice lacking protein kinase Cγ isoform. J Biol Chem 18:15409–15414.

Narita M, Mizoguchi H, Narita M, Nagase H, Suzuki, T, Tseng LF (2001b) Involvement of spinal protein kinase Cγ in the attenuation of opioid μ-receptor-mediated G-protein activation after chronic intrathecal administration of [D-Ala^2, N-MePhe^4, Gly-Ol^5]enkephalin. J Neurosci 21:3715–3720.

Ohsawa M, Narita M, Mizoguchi H, Suzuki T, Tseng LF (2000) Involvement of spinal protein kinase C in thermal hyperalgesia evoked by partial sciatic nerve ligation, but not by inflammation in the mouse. Eur J Pharmacol 403:81–85.

Pahl HL (1999) Activators and target genes of Rel/NF-κB transcription factors. Oncogene 18:6853–6866.

Pineau I, Lacroix S (2007) Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500: 267–285.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ (2001) Interleukin-1β-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410:471–475.

Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM (1988) cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 241:585–589.

Sweitzer SM, Colburn RW, Rutkowski M, DeLeo JA (1999) Acute peripheral inflammation induces moderate glial activation and spinal IL-1β expression that correlates with pain behavior in the rat. Brain Res 829:209–221.

Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 38:97–120.

Vanegas H, Schaible HG (2001) Prostaglandins and cyclooxygenases [correction of cyclooxygenases] in the spinal cord. Prog Neurobiol 64:327–363.

Yamada S, Kawate T, Sakamoto H, Aoki K, Hamada Y, Atsumi S (2006) Cyclo-oxygenase-2-immunoreactive neurons in the lumbar dorsal horn in a chicken acute inflammation model. Anat Sci Int 81:164–172.

Zeilhofer HU (2007) Prostanoids in nociception and pain. Biochem Pharmacol 73:165–174.

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Behavioural Pharmacology

Suppression of dopamine-related side effects of morphine by aripiprazole, a dopamine system stabilizer

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ABSTRACT

Dopamine receptor antagonists are commonly used to counter the adverse effects of opioids such as hallucinations, delusions and emesis. However, most of these agents themselves have side effects, including extrapyramidal symptoms. Here, we investigated the effect of the dopamine system stabilizer aripiprazole on morphine-induced dopamine-related actions in mice. Morphine-induced hyperlocomotion and reward were significantly suppressed by either the dopamine receptor antagonist prochlorperazine or aripiprazole. Catalepsy was observed with a high dose of prochlorperazine, but not with an even higher dose of aripiprazole. The increased level of dialysate dopamine in the nucleus accumbens stimulated by morphine was significantly decreased by pretreatment with aripiprazole. These results suggest that the co-administration of aripiprazole may be useful for reducing the severity of morphine-induced dopamine-related side effects.

1. Introduction

According to the World Health Organization (WHO) guidelines, morphine is considered the "gold standard" for the treatment of patients with moderate to severe pain due to cancer. However, the use of morphine for the treatment of cancer pain is sometimes accompanied by side effects, such as emesis, constipation, drowsiness and delirium (Aparasu et al., 1999; McNicol et al., 2003).

In the management of cancer pain, dopamine receptor antagonists such as haloperidol are used to protect against opioid-induced delirium (Ross and Alexander, 2001). In addition to delirium, dopamine receptor antagonists such as prochlorperazine have been considered by most clinicians to be the drug of choice to combat opioid-induced nausea and vomiting (Aparasu et al., 1999; McNicol et al., 2003).

These dopamine receptor antagonists are frequently associated with adverse effects, including extrapyramidal symptoms (Casey, 1995; Tonini et al., 2004). Therefore, new strategies for the prevention of opioid-induced delirium and emesis are required, along with a working knowledge of the proposed mechanism of drug action.

Aripiprazole is a novel dopamine D_2 receptor partial agonist that has a different pharmacological profile than currently marketed typical and atypical antipsychotics (Winans, 2003; Naber and Lambert, 2004). As a result, aripiprazole seems to provide a way to fine-tune the treatment of psychiatric disorders by maximizing the treatment effect while minimizing undesirable adverse events (Ohlsen and Pilowsky, 2005). Against this background, the present study was undertaken to evaluate whether aripiprazole could affect the severity of morphine-induced dopamine-related actions with fewer side effects.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

2.1. Animals

Male ICR mice (20–25 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) were used in the present study. Animals were housed in a room maintained at 22±1 °C with a 12 h light–dark cycle.

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Food and water were available ad libitum. Each animal was used only once.

2.2. Locomotor activity

The locomotor activity of mice was measured by an ambulometer as described previously (Narita et al., 1993). Briefly, a mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the mouse, was detected by three micro-switches. Total activity counts in each 10-min segment were automatically recorded for 180 min following the administration of saline (10 ml/kg, s.c.) or morphine (10 mg/kg, s.c.). Aripiprazole (3, 10, 20 mg/kg) or prochlorperazine (0.1, 0.3, 1 mg/kg) was co-administered with morphine s.c. 30 min prior to morphine treatment.

2.3. Place conditioning

Place-conditioning studies were conducted using a shuttle box (15×30×15 cm: $w \times l \times h$) that was made of an acrylic resin board and divided into two equal-sized compartments. One compartment was white with a textured floor and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test). The pre-conditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co. Ltd, Tokyo, Japan). Conditioning sessions (three for morphine: three for saline) were started the day after the pre-conditioning test and conducted once daily for 6 days. Groups of mice were pretreated with aripiprazole (10 or 20 mg/kg, s.c.), prochlorperazine (0.1or 0.3 mg/kg, s.c.) or their vehicle (saline or 5% dimethyl sulfoxide (DMSO), respectively) at 30 min before morphine or saline injection. Immediately after s.c.-injection of morphine at 5 mg/kg, these animals were placed in the compartment opposite that in which they had spent the most time in the pre-conditioning test for 1 h. On alternate days, these animals were treated with saline after the pretreatment with aripiprazole, prochlorperazine or their vehicle and placed in the other compartment for 1 h. On the day after the final conditioning session, a post-conditioning test that was identical to the pre-conditioning test was performed.

2.4. Horizontal bar test

To minimize the effects of arousal and stress, mice were handled gently and exposed to the testing site several times before measurements. Catalepsy was evaluated by placing the animal with both forelegs over a horizontal bar elevated 5 cm from the floor. The time (s) for which the mouse maintained this position was recorded for up to 60 s. Catalepsy was considered to be finished when the forepaw touched the floor or when the mouse climbed on the bar. A score was assigned to each test based on the duration of the cataleptic posture (score 1, between 15 and 29 s; score 2, between 30 and 59 s; score 3, 60 s or more).

2.5. In vivo microdialysis study and quantification of dopamine release

Stereotaxic surgery was performed under sodium pentobarbital (70 mg/kg, intraperitoneal injection) anesthesia. Mice were placed in a stereotaxic apparatus and the skull was exposed. A small hole was then made using a dental drill. A microdialysis probe (D-I-6-01; 1 mm membrane length; Eicom) was implanted into the nucleus accumbens (from bregma: anterior, +1.5 mm; lateral, −0.9 mm; ventral, −4.9 mm) according to the atlas of Paxinos and Franklin (Paxinos and Franklin, 1997). The microdialysis probe was fixed to the skull with cranioplastic cement. At 24 h after implantation, mice were placed in experimental cages (30 cm wide×30 cm long×30 cm high). The probe was perfused continuously at a flow rate of 2 ml/min with aCSF containing 0.9 mM $MgCl_2$, 147.0 mM NaCl, 4.0 mM KCl, and 1.2 mM $CaCl_2$. Outflow fractions were taken every 20 min. After three baseline fractions were collected, mice were given morphine (10 mg/kg, s.c.) or saline (1 ml/kg, s.c.). Aripiprazole (20 mg/kg, s.c.) or vehicle (saline; 1 ml/kg, s.c.) was injected 30 min before treatment with morphine or saline. For this experiment, dialysis samples were collected for 180 min after treatment with morphine or saline. Dialysis fractions were then analyzed using HPLC (Eicom) with ECD (HTEC-500; Eicom). Dopamine was separated by a column with a mobile phase containing sodium acetate (3.76 g/l), citric acid monohydrate (6.74 g/l), sodium 1-decane sulfonate (170 mg/l), EDTA (2Na; 5 mg/l), and 22% methanol. The mobile phase was delivered at a flow rate of 300 ml/min. Dopamine was identified according to the retention times of a dopamine standard, and the amounts of dopamine were quantified based on calculations using the peak areas.

2.6. Drugs

The drugs used in the present study were morphine hydrochloride (Daiichi-Sankyo Co., Tokyo, Japan), aripiprazole (Toronto Research Chemicals Inc., Ontario, Canada) and prochlorperazine (Sigma-Aldrich Co., St. Louis, MO, USA). Prochlorperazine was dissolved in 5% DMSO containing physiological saline, and morphine hydrochloride and aripiprazole was dissolved in physiological saline.

2.7. Statistical analysis

All data are presented as the mean±S.E.M. The statistical significance of differences between groups was assessed with Student's *t*-test or two-way ANOVA, followed by the Bonferroni/Dunnett test.

3. Results

3.1. Suppression of morphine-induced hyperlocomotion by pretreatment with either aripiprazole or prochlorperazine

Treatment with morphine (10 mg/kg, s.c.) produced a locomotor-enhancing effect. Groups of mice were pretreated with aripiprazole (3–20 mg/kg, s.c.) or prochlorperazine (0.1–1 mg/kg, s.c.) 30 min before morphine (10 mg/kg) injection. Pretreatment with either aripiprazole or prochlorperazine caused a dose-dependent suppression of morphine-induced hyperlocomotion (***$P<0.001$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ or ###$P<0.001$ vs. saline- or vehicle-morphine) (Fig. 1A, B). At the dose of aripiprazole which dramatically reduced the increase in locomotion caused by morphine, no hyper- or hypo-locomotion was observed compared to the saline group.

3.2. Suppression of morphine-induced place preference by pretreatment with aripiprazole or prochlorperazine

We next investigated whether pretreatment with aripiprazole (10 or 20 mg/kg) or prochlorperazine (0.1 or 0.3 mg/kg) could affect the rewarding effect of morphine. Pretreatment with aripiprazole or prochlorperazine inhibited the morphine-induced place preference (*$P<0.05$ or **$P<0.01$ vs. saline- or vehicle-saline, #$P<0.05$ or ##$P<0.01$ vs. saline- or vehicle-morphine) (Fig. 1C, D).

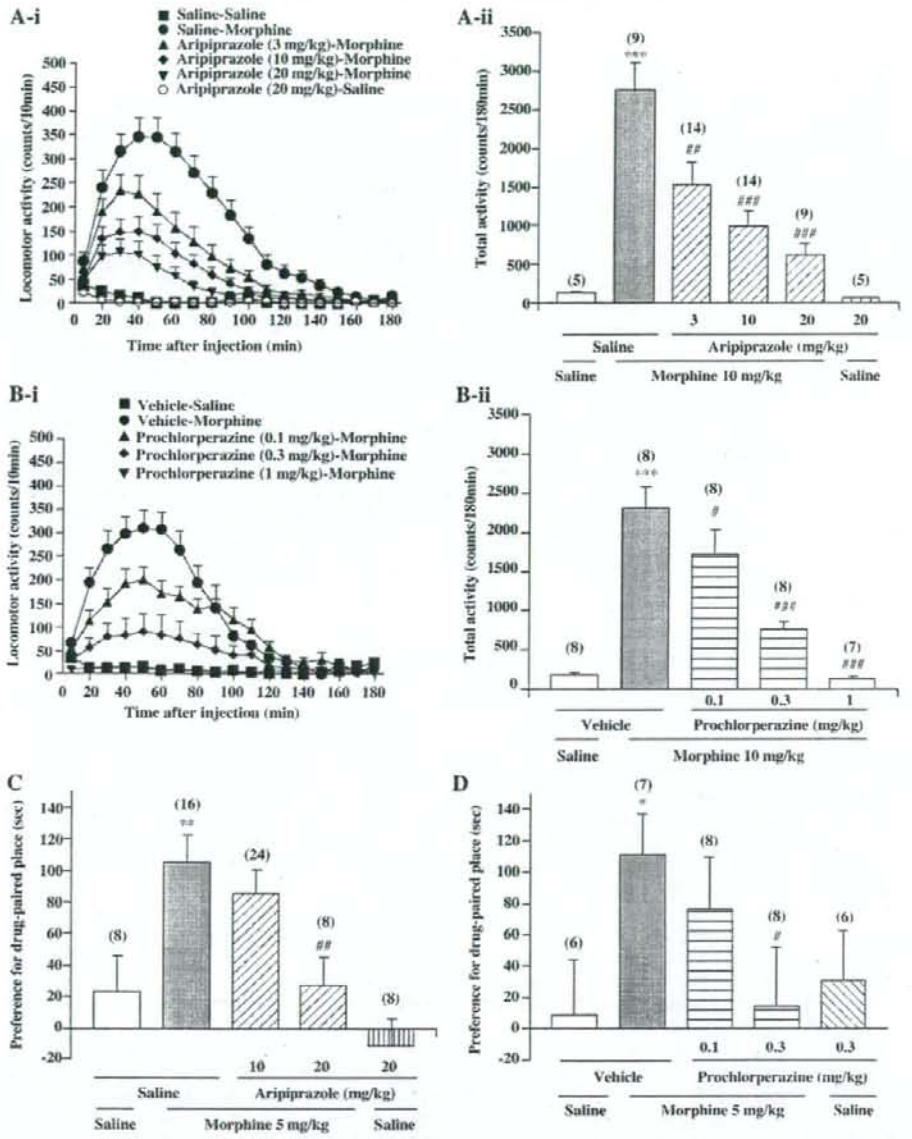

Fig. 1. Effects of aripiprazole or prochlorperazine on s.c. morphine-induced hyperlocomotion and conditioned place preference. (A) Groups of mice were pretreated with either aripiprazole (3–20 mg/kg, s.c.) or saline 30 min before morphine (10 mg/kg) injection. (B) Groups of mice were pretreated with prochlorperazine (0.1–1 mg/kg, s.c.) or vehicle (5% DMSO) 30 min before morphine (10 mg/kg) injection. Each column represents the mean total activity for 180 min with S.E.M. ***$P<0.001$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ or ###$P<0.001$ vs. saline- or vehicle-morphine. (C) Groups of mice were pretreated with either aripiprazole (10–20 mg/kg, s.c.) or saline 30 min before morphine (5 mg/kg) injection. (D) Groups of mice were pretreated with prochlorperazine (0.1–0.3 mg/kg, s.c.) or vehicle (5% DMSO) 30 min before morphine (5 mg/kg) injection. Immediately after s.c. injection of morphine, mice were placed and conditioned in either compartment for 1 h. Ordinate: mean differences between time spent in post-conditioning test and pre-conditioning test. Each column represents the mean with S.E.M. *$P<0.05$, **$P<0.01$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ vs. saline- or vehicle-morphine. Numbers in parentheses indicate the number of mice tested.

3.3. Effects of aripiprazole or prochlorperazine on catalepsy

Catalepsy values were obtained at 15, 30, 45 and 60 min after s.c. administration of saline, aripiprazole (3–40 mg/kg) or prochlorperazine (0.1–1 mg/kg). The catalepsy scores were not changed by a single s.c.-injection of aripiprazole (20 or 40 mg/kg), whereas a significantly high score was noted with prochlorperazine (1 mg/kg) (*$P<0.05$, **$P<0.01$ or ***$P<0.001$ vs. saline) (Fig. 2A, B).

3.4. Change in the increased dialysate dopamine level induced by morphine upon pretreatment with aripiprazole

In the microdialysis study, the dialysate dopamine levels in the mouse nucleus accumbens were markedly increased by s.c. injection of morphine at 10 mg/kg compared with that induced by saline ($F_{(1,66)}$= 15.47, $P<0.01$) (Fig. 2C). Under these conditions, the increased level of dialysate dopamine in the nucleus accumbens stimulated by morphine

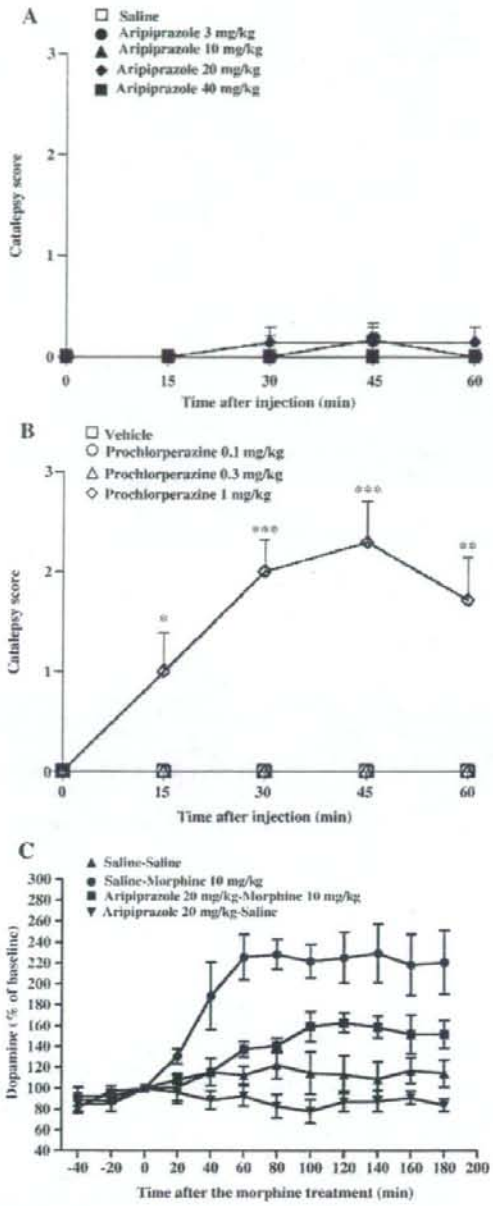

Fig. 2. Time-course of the catalepsy caused by aripiprazole (A) or prochlorperazine (B) in mice. Catalepsy values were obtained at 15, 30, 45 and 60 min after s.c. administration of saline, aripiprazole (3–40 mg/kg, s.c.), vehicle or prochlorperazine (0.1–1 mg/kg, s.c.), respectively. The horizontal bar test was performed by placing the forepaws of the mouse on a 5 cm-high bar. The time until the forepaw touched the floor or when the mouse climbed upon the bar was measured up to 60 s. A score was assigned to each test based on the duration of the cataleptic posture (score 1, between 15 and 29 s; score 2, between 30 and 59 s; score 3, 60 s or more). Each column represents the mean with S.E.M. of 6–7 mice. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. vehicle. (C) Effects of aripiprazole on the influence of s.c. morphine on the dialysate dopamine level in the nucleus accumbens. After baseline fractions were collected, mice were pretreated with either aripiprazole (20 mg/kg, s.c.) or saline 30 min before morphine (10 mg/kg) injection at time 0 to evoke the release of dopamine. Data are expressed as percentages of the corresponding baseline levels with S.E.M. for 4 mice. Saline–saline vs. saline–morphine 10 mg/kg, $F_{(1,68)}=15.47$, $P<0.01$; saline–morphine 10 mg/kg vs. aripiprazole 20 mg/kg-morphine 10 mg/kg, $F_{(1,66)}=9.836$, $p<0.05$.

was significantly decreased by pretreatment with aripiprazole at 20 mg/kg ($F_{(1,66)}=9.836$, $P<0.05$, saline-pretreated vs. aripiprazole-pretreated) (Fig. 2C). By itself, aripiprazole did not affect dopamine levels.

4. Discussion

Several clinical studies have suggested that undue anxiety about psychological dependence on opioids in cancer patients has caused

physicians and patients to use inadequate doses of opioids (WHO, 1996). It is widely accepted that the enhanced dopamine neuronal activity is a key factor in the development of psychological dependence on opioids (Narita et al., 2001).

Delirium is an organic psychiatric syndrome characterized by fluctuating consciousness and impaired perception, cognition, and behavior (Sipahimalani and Masand, 1998; Trzepacz, 2000). Exposure to opioids is associated with an increased risk of delirium in hospitalized cancer patients (Gaudreau et al., 2007). An excess of dopamine, glutamate, and norepinephrine, diminution of cholinergic function, and disturbances in gamma-aminobutyric acid (GABA) and serotonergic (5-hydroxytryptamine, 5-HT) activity have been implicated in its pathogenesis (Trzepacz, 2000).

In the management of cancer pain, nausea and vomiting are some of the most distressing adverse effects induced by opioids. Opioids induce emesis through various mechanisms: i.e., via stimulation of the chemoreceptor trigger zone in the brainstem and through enhanced vestibular sensitivity (Costello and Borison, 1977; Rubin and Winston, 1950). Although several agents that act on receptors in the chemoreceptor trigger zone are available, it has been determined that dopamine transmission is mainly responsible for opioid-induced nausea and vomiting.

Conventional antipsychotics that mainly act as dopamine D_2 receptor antagonists have been considered by most clinicians to be drugs of first choice for protecting against opioid-induced dopamine-related symptoms (Aparasu et al., 1999; Ross and Alexander, 2001; McNicol et al., 2003). However, most of these dopamine receptor antagonists are frequently associated with extrapyramidal symptoms (Tonini et al., 2004).

Aripiprazole is the first dopamine system stabilizer to mainly act as a dopamine D_2 receptor partial agonist, which is active against both positive and negative symptoms of schizophrenia. Uniquely, aripiprazole has a low propensity for extrapyramidal side effects, causes minimal weight gain or sedation, and produces no elevation in serum prolactin levels or prolongation of the QTc interval on electrocardiogram (Naber and Lambert, 2004). It has been clearly shown that aripiprazole has potent partial agonist activity at dopamine D_2 and 5-HT_{1A} receptors and antagonist activity at 5-HT_{2A} receptors (Li et al., 2004). In the present study, we first evaluated whether aripiprazole could affect the severity of morphine-induced dopamine-related actions.

It has been widely accepted that central dopaminergic systems contribute to hyperlocomotion induced by morphine in mice (Narita et al., 1993). As with the standard central dopamine D_2 receptor antagonist prochlorperazine, which has been widely administered as an anti-emetic drug for use with opioids, pretreatment with aripiprazole caused a dose-dependent suppression of morphine-induced hyperlocomotion. Similarly, we found that aripiprazole inhibited the morphine-induced place preference. Various studies have pointed out that the mesolimbic dopamine system is a critical pathway for the initiation of opioid-induced reinforcement (Funada and Shippenberg, 1996; Koob et al., 1998; Narita et al., 2001). In our biochemical experiments, aripiprazole failed to displace [tylosil-3,5-(3)H(N)]-[D-Ala(2), N-MePhe(4),Gly-ol(5)]enkephalin ([^{3}H]DAMGO) binding, which is a selective μ-opioid receptor ligand, in the mouse brain membrane, whereas it partially inhibited the guanosine 5′-o-(3-thio) triphosphate ([^{35}S]GTPγS) binding by dopamine but not morphine in the membrane of the mouse limbic forebrain including the nucleus accumbens, which is a terminus for the mesolimbic dopaminergic pathway (Narita et al., personal communication). In the present study, we found that pretreatment with aripiprazole caused a significant suppression of morphine-induced dopamine release in the nucleus accumbens. These findings suggest that a partial agonistic effect of aripiprazole on central dopamine D_2 receptors may suppress hyperlocomotion and the rewarding effects of morphine through both blockade of the firing of mesolimbic dopaminergic neurons in the ventral tegmental area and the competitive blockade of dopamine D_2 receptors in the nucleus accumbens.

In the present study, catalepsy was not observed after a single s.c. injection of aripiprazole at high doses, whereas it was produced by high doses of prochlorperazine. In a previous study, we demonstrated that aripiprazole at doses lower than those used here significantly suppressed morphine-induced retching or vomiting in ferrets (Shiokawa et al., 2007). Furthermore, aripiprazole did not appear to have any effect on morphine-induced antinociception in mice (Narita et al., personal communication). Taken together, these findings suggest that aripiprazole may have a low propensity for extrapyramidal side effects and may inhibit some of the distressing adverse effects of opioids.

In conclusion, we demonstrated here that morphine-induced hyperlocomotion, reward and dopamine release were significantly suppressed by pretreatment with the novel dopamine system stabilizer aripiprazole, whereas catalepsy was not produced by aripiprazole itself. Although more biochemical and clinical studies are required, we propose that the combination of aripiprazole with opioids may pave the way for a new strategy for controlling pain and suppressing the dopamine-related adverse effects of opioids.

References

Aparasu, R., McCoy, R.A., Weber, C., Mair, D., Parasuraman, T.V., 1999. Opioid-induced emesis among hospitalized nonsurgical patients: effect on pain and quality of life. J. Pain Symp. Manag. 18, 280–288.

Casey, D.E., 1995. Acute Extrapyramidal Syndromes. American Psychiatric Press, Inc., Washington DC.

Costello, D.J., Borison, H.L., 1977. Naloxone antagonizes narcotic self blockade of emesis in the cat. J. Pharmacol. Exp. Ther. 203, 222–230.

Funada, M., Shippenberg, T.S., 1996. Differential involvement of D1 and D2 dopamine receptors in the expression of morphine withdrawal signs in rats. Behav. Pharmacol. 7, 448–453.

Gaudreau, J.D., Gagnon, P., Roy, M.A., Harel, F., Tremblay, A., 2007. Opioid medications and longitudinal risk of delirium in hospitalized cancer patients. Cancer 109, 2365–2373.

Koob, G.F., Sanna, P.P., Bloom, F.E., 1998. Neuroscience of addiction. Neuron 21, 467–476.

Li, Z., Ichikawa, J., Dai, J., Meltzer, H.Y., 2004. Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. Eur. J. Pharmacol. 493, 75–83.

McNicol, E., Horowicz-Mehler, N., Fisk, R.A., Bennett, K., Gialeli-Goudas, M., Chew, P.W., Lau, J., Carr, D., 2003. Management of opioid side effects in cancer-related and chronic noncancer pain: a systematic review. J. Pain 4, 231–256.

Naber, D., Lambert, M., 2004. Aripiprazole: a new atypical antipsychotic with a different pharmacological mechanism. Prog. Neuropsychopharmacol. Biol. Psych. 28, 1213–1219.

Narita, M., Takahashi, Y., Takamori, K., Funada, M., Suzuki, T., Misawa, M., Nagase, H., 1993. Effects of kappa-agonist on the antinociception and locomotor enhancing action induced by morphine in mice. Jpn. J. Pharmacol. 62, 15–24.

Narita, M., Funada, M., Suzuki, T., 2001. Regulations of opioid dependence by opioid receptor types. Pharmacol. Ther. 89, 1–15.

Ohlsen, R.I., Pilowsky, L.S., 2005. The place of partial agonism in psychiatry: recent developments. J. Psychopharmacol. 19, 408–413.

Paxinos, G., Franklin, K., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press.

Ross, D.D., Alexander, C.S., 2001. Management of common symptoms in terminally ill patients: Part II. Constipation, delirium and dyspnea. Am. Fam. Phys. 64, 1019–1026.

Rubin, A., Winston, J., 1950. The role of the vestibular apparatus in the production of nausea and vomiting following the administration of morphine to man: clinical and experimental data including the effects of dramamine and benzedrine. J. Clin. Invest. 29, 1261–1266.

Shiokawa, M., Narita, M., Nakamura, A., Kurokawa, K., Inoue, T., Suzuki, T., 2007. Usefulness of the dopamine system-stabilizer aripiprazole for reducing morphine-induced emesis. Eur. J. Pharmacol. 570, 108–110.

Sipahimalani, A., Masand, P.S., 1998. Olanzapine in the treatment of delirium. Psychosomatics 39, 422–430.

Tonini, M., Cipollina, L., Poluzzi, E., Crema, F., Corazza, G.R., De Ponti, F., 2004. Review article: clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics. Aliment. Pharmacol. Ther. 19, 379–390.

Trzepacz, P.T., 2000. Is there a final common neural pathway in delirium? Focus on acetylcholine and dopamine. Semin. Clin. Neuropsychiatry 5, 132–148.

WHO, 1996. Cancer Pain Relief, 2nd ed. World Health Organization, Geneva.

Winans, E., 2003. Aripiprazole. Am. J. Health-Syst. Pharm. 60, 2437–2445.

プライマリ・ケア医の知っておきたい“ミニマム知識”
緩和ケアの普及について
—すべての医師が基本的な緩和ケアを実施できるように—

木澤　義之

Key words：緩和ケア，指導者研修会，緩和ケア教育

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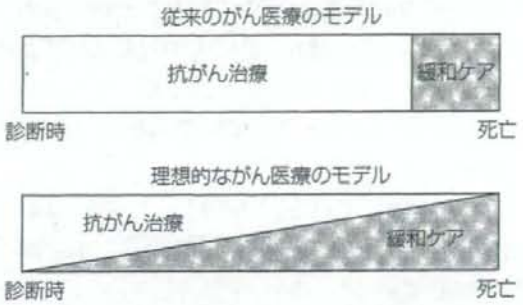
1．緩和ケアとは

WHOは2002年に，緩和ケアを以下のように定義している．「生命を脅かす疾患に伴う問題に直面する患者と家族に対し，疼痛や身体的，心理社会的，スピリチュアルな問題を早期から正確にアセスメントし解決することにより，苦痛の予防と軽減を図り，生活の質（QOL）を向上させるためのアプローチである．」従来緩和ケアは『看取りの医療』と取られがちであったが，体や心のつらさは，進行したがん患者だけではなく，がんと診断された早期の患者も抱えていることが稀ではない．図1に示すように，療養生活を可能な限り快適にするために，がんの痛みをはじめ，様々な苦痛を和らげる治療およびケア（緩和ケア）が，早期から行われることが重要であると考えられてきている．

わが国においても2007年に施行されたがん対策基本法の中で，療養生活の維持向上のために，早期から緩和ケアが適切に導入されることの重要性が述べられている．具体的に「緩和ケアが必要な時期」とは，患者・家族が何らかの苦痛や心配を訴えた時であり，その時が緩和ケアの開始時点である．緩和ケアを施行するかどうかは患者の状態が末期であるから，治療中であるからという状態によって決まるのではなく，患者に苦痛があるかどうかという点が重要である．



（「WHO：Cancer Pain Control」より抜粋）

図1．がん医療のモデル

2．基本的な緩和ケアの普及の現状

このように早期から緩和ケアを提供することの重要性が叫ばれてはいるが，わが国では，その普及が未だ十分ではなく，その一因として基本的な緩和ケアを行うための教育・支援体制が十分でないことが示唆されている．例えば，以下のようなデータがある[15]．

（1）がん性疼痛のガイドラインが十分に普及していない

WHO方式がん疼痛治療法は，がん性疼痛の国際的な治療ガイドラインであり，ガイドライン

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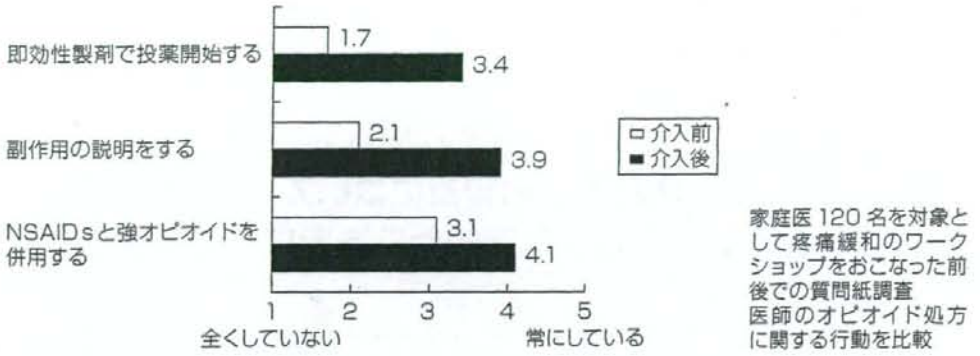


図2. 医師に対する教育的介入によるオピオイド処方の変化

に沿った治療を行うことで多くの患者において疼痛の緩和を行うことができることが知られている[1~3]. わが国においても, WHO方式鎮痛法のマニュアルが配布されるなど普及の努力が行われてきたが, 2008年の全国調査において, WHO方式がん疼痛治療法の「内容をよく知っている」「ある程度知っている」と回答した医師は約47%にとどまっており, 約28%の医師は「知らない」と解答している[4].

しかしながら, いくつかの介入研究によれば, 十分な教育体制をとってその支援を行うことで, オピオイドの処方など医師の行動を変化させることが可能であることが示唆されている[5](図2).

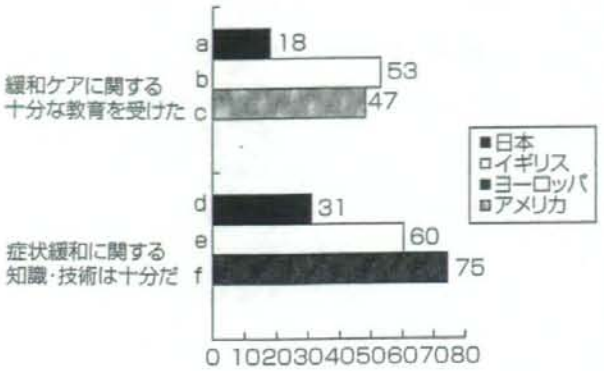
(2) 緩和ケアに関して十分な教育が行われていない

わが国で「緩和ケアに関して十分な教育を受けた」と回答した医師は約20%,「症状緩和に関する知識・技術が十分だ」と回答した医師は約30%にすぎず, 欧米と比して明らかに少ない[6~9]. 教育のプログラムとその組織的な普及が課題として挙げられる(図3).

3. 医師に対する緩和ケア教育—PEACEプロジェクト開始の背景

前述したような状況を速やかに改善させるために, 厚生労働省は, がん対策基本法に基づくがん対策推進基本計画(平成19年6月15日閣議決定)において「すべてのがん診療に携わる医師が研修等により, 緩和ケアについての基本的な知識を習得する」ことを目標として掲げた. これを受けて, がん診療に携わるすべての医師が緩和ケアについての基本的な知識を習得し, がん治療の初期段階から緩和ケアが提供されることを目的に, これら医師に対する緩和ケアの基本的な知識等を習得するための研修会を行うように各都道府県に厚生労働省健康局長通知「がん診療に携わる医師に対する緩和ケア研修会の開催指針(以下, 開催指針と略)」(平成20年4月1日付け健発第0401016号)が出された. その要点を表1に示す(表1). これを受けて, 日本緩和医療学会を中心に, 新たに「症状の評価とマネジメントを中心とした緩和ケアのための継続医学教育プログラム」(Palliative care Emphasis program on symptom management and Assessment for Continuous medical Education=PEACE)およびそれを用いた研修会(案)が開発された.

日本緩和医療学会は, 厚生労働省から「平成20年度がん医療に携わる医師に対する緩和ケア研修等事業」(平成20年5月9日付け健発0509004号)の委託を受けて, 関連団体等と協力して, このPEACEプログラムを用いた研修会である「がん診療に携わる医師に対する緩和ケア研修会」の実施および開催支援と, その指導者育成のための「緩和ケアおよび精神腫瘍学の基本教育に



a「症状緩和に関する十分な教育を受けた」に「とてもそう思う」,「そう思う」と回答した医師の割合（日本のがん専門病院 27 病院の医師 560 名を対象とした質問紙調査）
b「緩和ケアに関する十分な教育を受けた」に「とてもそう思う」「そう思う」と回答した医師の割合（イギリスの 6 地区から抽出した一般医師 492 名を対象とした質問紙調査）
c「疼痛緩和に関して適切な教育を受けた」に「非常に適切」「適切」と回答した医師の割合（アメリカ New Hampshire の無作為抽出で選定された医師 63 名を対象とした質問紙調査）
d「症状緩和に関する知識・技術が十分である」に「とてもそう思う」,「そう思う」と回答した医師の割合（a と同じ調査対象）
e「症状緩和に自信がある」と回答した医師の割合（b と同じ調査対象）
f「症状緩和の専門家としての知識がある」に「とてもそう思う」「そう思う」と回答した医師の割合（European Society of Medical Oncology に所属する医師 895 名を対象とした質問紙調査）

図 3. 緩和医療に関する医師の自信

表 1. 緩和ケア研修会標準プログラムの要件

一般型研修会
①研修時間は全体で 12 時間以上，2 日以上に渡ること
②プレテストとその解説を行うこと
③アイスブレーキングの時間を設けること
④がん性疼痛の講義は，基礎，WHO 方式について，治療法を含むこと
⑤④と別にがん性疼痛のワークショップを 180 分以上行うこと
（疼痛症例のグループ討議＋オピオイド処方時の患者説明のロールプレイ）
⑥呼吸困難の講義，消化器症状の講義を含むこと
⑦精神症状（不安・抑うつ・せん妄）の講義を行うこと
⑧コミュニケーションの講義を行うこと
⑨コミュニケーションのワークショップを 90 分以上行うこと
（bad news の伝え方のグループ討議＋同ロールプレイ）
※⑧と⑨は合わせて 180 分（2 単位）以上で，同じ日に行われなければならない
⑩ワークショップ（疼痛・コミュニケーション）は，原則として 6 ～ 10 名程度のグループに分かれること
⑪地域の状況をふまえつつ，以下の内容を含むこと
1）全人的な緩和ケアの要点
2）放射線・神経ブロックの適応，専門的緩和ケアへの依頼の要点
3）療養場所の選択と地域連携
4）在宅における緩和ケア
※ワークショップ以外の講義には時間の条件設定がないが，概ね単位型の【1 単位＝ 90 分以上】の時間設定に沿うのがよいと思われる

関する指導者研修会」を軸とした緩和ケア教育プロジェクトを「日本緩和医療学会PEACEプロジェクト」として実施している．

（1）PEACEプロジェクトの目的

PEACEプロジェクトの目的は，基本的な緩和ケアの啓発と普及，教育を通して，すべてのが

表2. PEACEプログラムで用意されているプレゼンテーション

M-1：	緩和ケア研修会の開催にあたって
M-2：	緩和ケア概論
M-3：	がん性疼痛の評価と治療
M-4a：	がん性疼痛事例検討
M-5：	オピオイドを開始するとき
M-6a：	呼吸困難
M-6b：	消化器症状（嘔気・嘔吐）
M-7a：	気持ちのつらさ
M-7b：	せん妄
M-8：	コミュニケーション
M-9：	地域連携と治療・療養の場の選択
T-1：	緩和ケア研修会とPEACEプロジェクト
T-2：	教育技法
T-3：	アイス・ブレイキング

ん診療に携わる医師が緩和ケアについての基本的な知識を習得することを推進し，国民がその療養場所にかかわらず質の高い緩和ケアを受けることができるようにすることである．従って，開発されたPEACEプログラムも緩和ケアに専門に従事する医療従事者に対するものではなく，がん診療に携わるすべての医師に必要な緩和ケアが学習できるように組み立てられている．

（2）PEACEプログラムの紹介

開発された緩和ケア研修会用のPEACEプログラムは2日間にわたる計780分のプログラムで，厚生労働省から出された開催指針で定める「緩和ケア研修会標準プログラム」に準拠している．本プログラムは，一般型研修会プログラム例，アイス・ブレイキング，緩和ケアの概論，症状アセスメント，がん性疼痛をはじめとする身体症状の緩和，そして地域連携に関する研修からなっており現在作成，公開されているのは以下の通りである[11]．

1）エンドユーザータイムテーブル

2）PEACEプレゼンテーション（PDF形式で公開，14モジュール，表2参照）

3）参加者ハンドブック

なお，本PEACEプログラムは，日本医師会発行の『がん緩和ケアガイドブック2008年版』[12]（http://www.med.or.jp/etc/cancer.htmlからダウンロード可能）に準拠して作成されており，研修会を行う際のテキストとして本ガイドブックの使用が推奨される．またより詳細なものとしては，OPTIM（緩和ケアプログラムによる地域介入研究）のステップ緩和ケア，および患者家族用パンフレット[13]（http://gankanwa.jp/からダウンロード可能）ともその内容を一致させており，あわせて参考資料として活用することが可能である．また，プレテストおよびポストテストの実施については，日本ホスピス緩和ケア研究振興財団による『緩和ケア専従医のための自己学習プログラム』[14]に多肢選択式の問題が多数収載され，詳細な解説がなされているため参考にされたい．

（http://www.hospat.or.jp/program.htmlよりダウンロード可能）

（3）PEACEプロジェクトの構造

本プロジェクトは2つの大きな柱からなっている．それは，指導者研修会（『緩和ケアの基本教育に関する指導者研修会』および『精神腫瘍学の基本教育に関する指導者研修会』）の実施と全国各地における緩和ケア研修会の開催支援である．普及の手段として，まず各地方やがん診療拠点病院で研修会を開催する指導者を育成し，教育マテリアルを整備した上で育成した指導者が地域で緩和ケア研修会を開催することを支援するという構造をとっている（図4）．各々詳細を述べる．

1）指導者研修会

指導者研修会の実施は，本プロジェクトの根幹をなすものである．指導者研修会は，緩和ケア研修会の企画，運営，痛みをはじめとする身体症状の教育を担当する指導者に対する『緩和ケアの基本教育に関する指導者研修会（2泊3日）』と，精神症状や気持ちのつらさ，コミュニケーションの教育を担当する指導者に対する『精神腫瘍学の基本教育に関する指導者研修会（1泊2日）』に分けて行われる．双方のプログラム