Selective COX-2 inhibitor celecoxib prevents experimental autoimmune encephalomyelitis through COX-2-independent pathway

Katsuichi Miyamoto,1,2 Sachiko Miyake,1 Miho Mizuno,1 Nobuyuki Oka,3 Susumu Kusunoki2 and Takashi Yamamura1

1Department of Immunology, National Institute of Neuroscience, NCNP, Tokyo, 2Department of Neurology, Kinki University School of Medicine, Osaka and 3Department of Rehabilitation Medicine, Minami-kyoto National Hospital, Kyoto, Japan

Correspondence to: Sachiko Miyake, Department of Immunology, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187-8502, Japan
E-mail: miyake@ncnp.go.jp

Cyclooxygenase (COX) is a key enzyme of arachidonic acid metabolism and exists as two distinct isoforms. COX-1 is constitutively expressed in most tissues, whereas COX-2 is inducibly expressed at the site of inflammation. Selective inhibitors of COX-2 have been developed and have been used as anti-inflammatory agents. Here, we show that a new-generation COX-2 inhibitor, celecoxib, inhibited experimental autoimmune encephalomyelitis (EAE). Celecoxib, but not other COX-2 inhibitors such as nimesulid, prevented myelin oligodendrocyte glycoprotein (MOG) induced EAE when administrated orally on the day of disease induction. Moreover, celecoxib inhibited EAE in COX-2-deficient mice, indicating that celecoxib inhibited EAE in a COX-2-independent manner. In celecoxib-treated mice, interferon-γ (IFN-γ) production from MOG-specific T cells was reduced and MOG-specific IgG1 was elevated compared with vehicle-treated mice. Infiltration of inflammatory cells into the central nervous system and the expression of adhesion molecules, P-selectin and intercellular adhesion molecule-1 (ICAM-1), and a chemokine, monocyte chemoattractant peptide-1 (MCP-1), were inhibited when mice were treated with celecoxib. These results suggest that celecoxib may be useful as a new additional therapeutic agent for multiple sclerosis.

Keywords: COX-2 inhibitor; celecoxib; experimental autoimmune encephalomyelitis; multiple sclerosis

Abbreviations: CMC = carboxymethylcellulose; COX = cyclooxygenase; EAN = experimental autoimmune neuritis; EAE = experimental autoimmune encephalomyelitis; ELISA = enzyme-linked immunosorbent assay; ICAM-1 = intercellular adhesion molecule-1; IFN = interferon; IL = interleukin; LN = lymph node; MCP-1 = monocyte chemoattractant peptide-1; MOG = myelin oligodendrocyte glycoprotein; PBS = phosphate-buffered saline


Introduction
Cyclooxygenase (COX) catalyses the conversion of arachidonic acid to prostaglandins and has two isoforms, COX-1 and COX-2 (Vane et al., 1994; Warner and Mitchell, 2004). COX-1 is constitutively expressed in most tissues and produces prostaglandins involved in maintenance of the gastric mucosa, regulation of renal blood flow and platelet aggregation. On the other hand, COX-2 is inducibly expressed in cells involved in inflammation and in neoplastic tissues by proinflammatory and mitogenic stimuli, and is primarily responsible for the synthesis of prostanooids involved in acute and chronic inflammation (Xie et al., 1997). COX-2 therefore appears to be a suitable target for the anti-inflammatory effects of non-steroidal anti inflammatory drugs. These findings have provided the rationale for the development of selective inhibitors of COX-2.

Celecoxib is a new generation of highly specific COX-2 inhibitors that have been approved for the treatment of rheumatoid arthritis and other inflammatory diseases. The selectivity of COX-2 inhibition is much higher than traditional COX-2 inhibitors (Penning et al., 1997). Furthermore, celecoxib has been shown to exert a potent anti-tumour effect. Interestingly, the anti-tumour effect by celecoxib
has been reported via both COX-2-dependent and COX-2-independent mechanisms (Grosch et al., 2001). For example, cell cycle arrest and apoptosis of various kinds of cells induced by celecoxib appeared to be COX-2-independent effects (Hsu et al., 2000; Arico et al., 2002; Liu et al., 2004).

Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model for multiple sclerosis that can be induced by immunization with myelin antigens such as myelin oligodendrocyte glycoprotein (MOG). EAE is mediated primarily by CD4+ Th1 T cells producing interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) (Nicholson and Kuchroo, 1996; Kumar et al., 1997; Zhang et al., 1997). COX-2 is expressed in neurons and endothelial cells in healthy brain. In rats with EAE, the expression of COX-2 was reported to be upregulated in endothelial cells in inflammatory lesions. In addition, non-selective COX-2 inhibitors have been reported to have moderately ameliorate EAE (Prosigel et al., 1989; Weber et al., 1991; Simmons et al., 1992), suggesting that COX-2 may have an important role in the pathogenesis of EAE (Deininger and Schlesener, 1999). Furthermore, we recently demonstrated that COX-2 inhibitors suppress experimental autoimmune neuritis (EAN), a model of Guillain–Barré syndrome, which is also characterized as a CD4+-Th1 T-cell-mediated autoimmune neurological disease model similar to EAE (Miyamoto et al., 1998, 1999, 2002). These findings led us to investigate the effect of COX-2 inhibitors on EAE.

In the present study, we found that celecoxib greatly suppressed EAE in comparison with traditional COX-2 inhibitors. Furthermore, we have demonstrated that celecoxib inhibited EAE by inhibiting Th1 response of autoreactive T cells and that this inhibition was COX-2-independent. Finally, we demonstrated that celecoxib prevented cell entry into the CNS in association with the inhibition of the expression of P-selectin, intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant peptide-1 (MCP-1). These results highlighted the COX-2-independent therapeutic potential of celecoxib for multiple sclerosis.

Material and methods

Mouse

Wild-type C57BL/6 (B6) mice were purchased from Clea Japan (Tokyo, Japan). COX-2-deficient mice (COX-2−/−) have been backcrossed to B6 background for more than five generations and were purchased from Taconic (Germantown, NY, USA). These mice were maintained under specific pathogen-free conditions.

Induction of EAE

For induction of EAE, mice were immunized (5–10 mice per group) subcutaneously in flanks with 100 μg of MOG35–55 peptide (MEVG-WYRSPFSRVHLYRNGK) in 0.1 ml phosphate-buffered saline (PBS) and 0.1 ml complete Freund's adjuvant (CFA) containing 1 mg Mycobacterium tuberculosis H37Ra (Difco Laboratories, Detroit, MI, USA) and were injected intravenously with 200 ng of pertussis toxin (List Biological Laboratories, Campbell, CA, USA) on the day of immunization and 2 days later.

Clinical assessment of EAE

EAE was scored on the following scale: 0 = no clinical signs; 1 = partial loss of tail tonicity; 2 = completely limp tail and abnormal gait; 3 = partial hindlimb paralysis; 4 = complete hindlimb paralysis; and 5 = fore- and hindlimb paralysis or moribund state.

Treatment with COX-2 inhibitors

Mice were orally administered 5 μg/g of COX-2 inhibitor, celecoxib (Searle, St Louis, MO, USA) (Penning et al., 1997), nimesulid (Nakarai Tesque, Kyoto, Japan) (Nakatsuji et al., 1996), or indomethacin (Nakarai Tesque) in 0.5% carboxymethylcellulose (CMC) via a feeding cannula every 2 days. Control mice were orally administered vehicle (0.5% CMC) alone.

Measurement of MOG35–55-specific IgG1 and IgG2a titres

Enzyme-linked immunosorbent assay (ELISA) plates (Sumitomo, Tokyo, Japan) were coated with 10 μg/ml MOG35–55 in PBS overnight at 4°C. After blocking with 2% bovine serum albumin (BSA) in PBS, different dilutions of the serum from animals at Day 30 after immunization, or normal mice or PBS were added to the plate. MOG35–55-specific antibodies were detected using biotin-labelled anti-IgG1 and anti-IgG2a antibodies (Vector Laboratories, Burlingame, CA, USA). After adding streptavidin-peroxidase (BD Biosciences, San Jose, CA, USA) and a substrate, plates were read at OD450 values.

MOG35–55-specific T-cell proliferation assay

On Day 11 after immunization with MOG35–55, draining lymph nodes (LN) were harvested and single cell suspensions were prepared. Cells were cultured in RPMI1640 medium (Gibco, Grand Island, NY, USA) supplemented with 5 × 10−5 M 2-mercaptoethanol, 2 mM l-glutamine, 100 U/ml penicillin and streptomycin and 1% autologous mouse serum, and seeded onto 96-well flat-bottom plates (1 × 105 cells/well). The cells were stimulated with peptide for 72 h at 37°C in a humidified air condition with 5% CO2. To measure cellular proliferation, 1H]-thymidine was added (1 μCi/well) and uptake of the radioisotope during the final 18 h of culture was counted with a beta-1205 counter (Pharmacia, Uppsala, Sweden). To evaluate proliferative responses of LN cells to peptide, we determined the Δc.p.m. value for cells in each well by subtracting the background c.p.m.

Detection of cytokines and chemokine

LN cells from the MOG35–55 immunized mice were cultured in the standard medium in 96-well flat-bottom plates at 1 × 105/well for 48 h in the presence of the different concentrations of MOG35–55. The concentrations of IFN-γ, interleukin-4 (IL-4) and IL-10 in the supernatants were measured by using a sandwich ELISA following the protocol provided by BD Biosciences. A chemokine, MCP-1, in the serum from mice on Day 7, 10 and 14 after induction of EAE was also measured by using a sandwich ELISA following the protocol provided by BD Biosciences. All reagents, including recombinant mouse cytokines, chemokine and antibodies were purchased from BD Biosciences.
Analysis of infiltrating cells isolated from CNS

Mice were anaesthetized with diethyl ether on Day 14 after induction of EAE. After perfusion with PBS, brain and spinal cord were removed and homogenized. After washing with PBS, mononuclear cells were isolated using Ficoll gradient (Amersham Biosciences, Piscataway, NJ, USA) (Krakowski et al., 1997). The cells were stained with APC-labelled anti-CD3 antibody, fluorescein isothiocyanate (FITC) labelled anti-CD4 or CD8 or CD19 antibody (BD Biosciences) and were analysed by flow cytometer (BD FACS Calibur). Apoptosis of lymphocytes was analysed by using Annexin-5 apoptosis kit (BD Biosciences).

Pathological analysis

The brain and spinal cord were removed on Day 7, 10 and 14 after induction of EAE. Ten-micrometre frozen sections were fixed with acetone and stained with haematoxylin and eosin (HE), Luxol fast blue or antibodies of adhesion molecule ICAM-1 (CD54), vascular cell adhesion molecule-1 (VCAM-1: CD106), E-selectin (CD62E) and P-selectin (CD62P) (BD Biosciences), following the protocol provided by BD Biosciences.

Statistics

For statistic analysis, non-parametric Mann–Whitney U-test was used to calculate significant levels for all measurements. Values of $P < 0.05$ were considered statistically significant.

Results

Celecoxib inhibits EAE

To examine the effect of celecoxib on the development of EAE, we first administered celecoxib at the time of immunization with MOG35–55. Oral administration of celecoxib reduced the incidence of disease and suppressed maximum EAE score and cumulative score compared with the control group (Fig. 1A, Table 1). Histological comparison between the thoracic region of the spinal cord demonstrated reduced monocyte infiltration and demyelination in cerebroxib-treated mice compared with vehicle-treated mice (Fig. 2A–D). Celecoxib was also effective in reducing the severity of disease when administered at Day 8

![Fig. 1](image-url) Effect of celecoxib on actively induced EAE. EAE was induced in female B6 mice by immunization with MOG35–55 in CFA as described in Material and methods. (A) Mice were orally administered 5 $\mu$g/g (closed circles) or 10 $\mu$g/g (open diamond) of celecoxib starting from the day of the immunization, or with 5 $\mu$g/g of celecoxib starting from 8 days after the immunization (open circles). Control mice were administered vehicle alone (closed squares). Statistical analysis is shown in Table 1. (B) Mice were orally administered 5 $\mu$g/g of celecoxib (closed circles) or nimesulid (open triangle) or indomethacin (crosses) every 2 days from the day of EAE induction. Control mice were administered vehicle alone (closed squares). Statistical analysis is shown in Table 2. One representative experiment of two independent experiments is expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Table 1 Clinical scores of EAE treated with celecoxib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. score</td>
</tr>
<tr>
<td>Control (CMC)</td>
</tr>
<tr>
<td>Celecoxib 10 $\mu$g/g</td>
</tr>
<tr>
<td>Celecoxib 5 $\mu$g/g</td>
</tr>
<tr>
<td>Celecoxib 5 $\mu$g/g (from Day 8)</td>
</tr>
</tbody>
</table>

Four groups of mice were immunized with MOG35–55 peptide for induction of EAE. The control CMC solution, 5 or 10 $\mu$g/g of celecoxib diluted in CMC, was orally injected via a cannula every 2 days starting from Day 0 or 8 after induction of EAE. Mean ± SEM of the following parameters are shown: maximum score of EAE (Max. score), the days of EAE onset, incidence of paralysed mice among sensitized mice (Incidence) and summation of the clinical scores from Day 0 to 30 (Cumulative score). *P < 0.05 versus control.
post-EAE-induction. Although indomethacin suppressed EAE to some extent, all mice died around Day 30 after immunization owing to intestinal ulcer. In contrast, oral administration of nimesulid, another COX-2 inhibitor, did not suppress either the incidence or the severity of EAE (Fig. 1B). Composite data from experiments is shown in Tables 1 and 2.

**Celecoxib inhibits MOG-specific Th1 response**

To determine the mechanisms by which celecoxib inhibits EAE, we examined the level of MOG-specific IgG1 and IgG2a in the serum samples collected from individual EAE-induced mice on Day 30. It is generally accepted that elevation of antigen-specific IgG2a antibody results from augmentation of a Th1 immune response to the antigen, whereas a higher level of IgG1 antibody would reflect a stronger Th2 response to the antigen. There was a significant elevation of the level of MOG35–55-specific IgG1 and a slight reduction in the level of MOG-specific IgG2a in celecoxib-treated group compared with vehicle-treated group (Fig. 3A). In contrast, there was no significant difference in the level of either IgG1 or IgG2a in nimesulid-treated mice compared with vehicle-treated group (Fig. 3B).

To further investigate the response of T cells to MOG35–55 in celecoxib-treated mice, we examined the proliferative response and cytokine production of draining LN cells in vitro. Mice were immunized with MOG35–55 and were administered celecoxib or vehicle on the day of immunization. Ten days after immunization, draining LN cells were collected and cultured with MOG35–55 peptide. As shown in Fig. 4A, there was no significant difference in a proliferative response of MOG-reactive T cells between celecoxib-treated and vehicle-treated groups. We next examined the levels of cytokines in the culture supernatant by ELISA. The level of IFN-γ was reduced in the culture supernatants of LN cells obtained from mice treated with celecoxib compared with that from control mice (Fig. 4B). IL-4 and IL-10 were not detected in either culture supernatant. These results indicate that celecoxib reduces Th1 cytokine production from MOG-reactive T cells.

**Celecoxib prevents EAE even in COX-2-deficient mice**

Since another COX-2 inhibitor, nimesulid, did not have the inhibitory effect on EAE, we examined whether celecoxib could inhibit EAE in COX-2-deficient mice. As shown in Fig. 5A, the maximum EAE score, the day of onset and the severity of EAE were not significantly different between COX-2−/− and wild-type mice. Administration of celecoxib prevented the development of EAE in COX-2−/− mice as well as in wild-type mice. Consistent with the severity of EAE, the levels of MOG-specific IgG1 and IgG2a in COX-2−/− mice were not different compared with wild-type B6 mice (Fig. 5B). Moreover, celecoxib treatment increased the level of MOG-specific IgG1 even in COX-2−/− mice, resulting in the elevation of IgG1 : IgG2a ratio similar to that in wild-type mice (CMC = 0.29, celecoxib = 3.00) and COX-2−/− mice (CMC = 0.42, celecoxib = 2.52). These results indicate that the effect on the inhibition of EAE...
and Th1 response by celecoxib is mediated by a COX-2-independent pathway (Table 3)

<table>
<thead>
<tr>
<th></th>
<th>Max. score</th>
<th>Day of onset</th>
<th>Incidence (%)</th>
<th>Cumulative score</th>
<th>Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CMC)</td>
<td>3.05 ± 0.20</td>
<td>13.10 ± 1.16</td>
<td>100 (10/10)</td>
<td>26.47 ± 5.13</td>
<td>10 (1/10)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1.02 ± 0.53*</td>
<td>14.30 ± 1.77</td>
<td>90 (9/10)</td>
<td>7.58 ± 6.72*</td>
<td>0 (0/10)</td>
</tr>
<tr>
<td>Nimesulid</td>
<td>2.54 ± 0.68</td>
<td>13.50 ± 1.56</td>
<td>100 (10/10)</td>
<td>22.15 ± 4.75</td>
<td>0 (0/10)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.70 ± 0.83</td>
<td>13.90 ± 1.93</td>
<td>100 (10/10)</td>
<td>15.21 ± 3.89</td>
<td>100 (10/10)*</td>
</tr>
</tbody>
</table>

Each mouse was immunized with MOG35-55 peptide for induction of EAE. The control CMC solution, or 5 µg/g of drugs diluted in CMC, was orally administered via a cannula every other day. Mean ± SEM of the following parameters are shown: maximum score of EAE (Max. score), the days of EAE onset, incidence of paralysed mice among sensitized rats (Incidence), summation of the clinical scores from Day 0 to 30 (Cumulative score) and the incidence of death during EAE (Death). *P < 0.05 versus control.

Celecoxib suppresses the expression of adhesion molecules and a chemokine related to cell infiltration into CNS

For the recruitment of autoreactive T cells into the brain through the blood–brain barrier (BBB), some adhesion molecules such as ICAM-1, VCAM-1 and P-selectin, and chemokines such as MCP-1 are required (Engelhardt et al., 1997; Hofmann et al., 2002). We performed an immunohistostaining of sliced brain sections from mice with EAE using antibodies against adhesion molecules. ICAM-1, VCAM-1 and P-selectin (Fig. 6A, C and E) were expressed on choroid plexus in the brain obtained from EAE-induced mice. In contrast, in brains obtained from celecoxib-treated mice, the expression level of P-selectin and ICAM-1 was lower compared with the control (Fig. 6B, D and F). In addition, we examined the level of MCP-1, which is an important chemokine involved in recruiting autoreactive T cells into the brain. As shown in Table 5, the level of MCP-1 in the serum obtained from
celecoxib-treated mice was significantly lower compared with that obtained from vehicle-treated mice. These findings suggested that celecoxib inhibits an infiltration of immune-mediated cells into CNS through the BBB by suppression of P-selectin, ICAM-1 and MCP-1.

**Discussion**

In the present study, we have demonstrated that a new-generation selective COX-2 inhibitor, celecoxib, strongly inhibited the development of EAE as compared with vehicle treatment or a traditional COX-2 inhibitor, nimesulid. The
Inhibitory effect on EAE by celecoxib was also evident in COX-2-deficient mice, indicating that celecoxib suppressed EAE in a COX-2-independent mechanism. In celecoxib-treated mice, MOG-specific Th1 responses were reduced and infiltration of immune cells was significantly inhibited compared with vehicle-treated mice, which were associated with lower expression of ICAM-1 and P-selectin on the choroid plexus in the brain.

Since EAE is an autoimmune inflammatory disease, administering COX-2 inhibitor was expected to inhibit disease as well as other COX inhibitors. Recently, Muthian et al. (2006) showed that some COX-2 inhibitors such as NS398 and LM01 suppressed EAE, when administered intraperitoneally every other day. In their study, they observed the inhibitory effect of nimesulid on EAE when orally administered every 2 days using the same conditions in which celecoxib exhibited a strong inhibitory effect. The route and timing of administration might be critical to modulate diseases. The inhibitory effect mediated by celecoxib was stronger compared with other COX inhibitors, suggesting that different mechanisms might be occurring in addition to the suppression of production of prostanooids that occurred at sites of disease and inflammation. In fact, COX-2 was not required for the celecoxib-mediated inhibitory effect on EAE. Recent studies have suggested that COX-2-independent pathways may contribute to celecoxib-mediated anti-tumour or anti-arthritic effect through enhanced apoptosis of tumour cells or synovial cells (Kusunoki et al., 2002; Shishodia et al., 2004). In our study, enhancing apoptosis of immune cells was not detected, indicating that different COX-2-independent mechanisms might be important for celecoxib-mediated inhibition of EAE. We observed that celecoxib treatment inhibited Th1 responses of MOG-reactive T cells. In the regulation of Th1/Th2 responses, prostaglandin E2 synthesized by COX has been reported to suppress IL-2 and IFN-γ production by a Th1 clone (Snijdewint et al., 1993). In addition, Meyer et al. (2003) reported that administration of COX-2 inhibitor, NS398, increased Helicobacter-stimulated IL-12 and IFN-γ production, suggesting that COX-2 inhibition resulted in enhanced Th1 responses. In contrast, celecoxib inhibited Th1 responses of autoreactive T cells. Therefore, this COX-2-independent effect on immune system may be a mechanism to explain why celecoxib suppresses EAE to a greater degree compared with that of other COX-2 inhibitors. Allonza et al. (2006) reported that

---

**Table 3** Clinical scores of EAE in COX-2-deficient mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Treatment</th>
<th>Max. score</th>
<th>Day of onset</th>
<th>Incidence (%)</th>
<th>Cumulative score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>CMC</td>
<td>3.54 ± 0.28</td>
<td>12.60 ± 1.15</td>
<td>100 (10/10)</td>
<td>24.85 ± 6.37</td>
</tr>
<tr>
<td></td>
<td>Celecoxib</td>
<td>1.13 ± 0.39</td>
<td>13.20 ± 1.80</td>
<td>80 (8/10)</td>
<td>6.29 ± 4.02*</td>
</tr>
<tr>
<td>COX-2-/-</td>
<td>CMC</td>
<td>3.75 ± 0.44</td>
<td>12.78 ± 1.57</td>
<td>100 (8/8)</td>
<td>29.88 ± 5.62*</td>
</tr>
<tr>
<td></td>
<td>Celecoxib</td>
<td>1.46 ± 0.51</td>
<td>14.13 ± 1.96</td>
<td>87.5 (7/8)</td>
<td>5.39 ± 3.36*</td>
</tr>
</tbody>
</table>

Wild-type and COX-2-/- mice were immunized with MOG35–55 peptide to induce EAE. The control CMC solution, or 5 μg/g of celecoxib diluted in CMC, was administered every other day. Mean ± SEM of the following parameters are shown: maximum score of EAE (Max. score), the days of EAE onset, incidence of paralysed mice among sensitized mice (Incidence) and summation of the clinical scores from Day 0 to 30 (Cumulative score). *P < 0.05 versus control.

**Table 4** Cell infiltration into the CNS of EAE-induced mice

<table>
<thead>
<tr>
<th>EAE mice</th>
<th>Mononuclear cell</th>
<th>CD3+ cell</th>
<th>CD4+ cell</th>
<th>CD19+ cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CMC)</td>
<td>667 ± 176</td>
<td>203 ± 69</td>
<td>158 ± 50</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>90 ± 57*</td>
<td>12 ± 8*</td>
<td>9 ± 5*</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Naive mice</td>
<td>20 ± 6</td>
<td>5 ± 2</td>
<td>3 ± 2</td>
<td>1 ± 0</td>
</tr>
</tbody>
</table>

CNS tissues from each group mouse were homogenized on Day 18 after immunization with MOG35–55 peptide. Mononuclear cells were isolated by Percoll solution. The cells were stained with cell markers and analysed by flow cytometer. Mean ± SEM of cell number ($10^3$ cells/mouse) is shown. Representative data of two independent experiments are shown (n = 5 for each group). *P < 0.05 versus control.
Celecoxib inhibits IL-12 αβ and β2 folding and secretion in association with the increased interaction of IL-12 with reticulin, an endoplasmic reticulum-resident chaperone in retention of misfolded cargo proteins, while blocking interaction with Erp44. They also demonstrated that an analogue of celecoxib lacking the COX-2 inhibitor activity showed identical effects to that of celecoxib on folding and secretion of IL-12, indicating that the effect is COX-2-independent. Since IL-12 is a key cytokine to provoke Th1 immune response, reduction in MOG-specific Th1 response is consistent with these previous findings.

The infiltration of immune cells in the CNS was significantly inhibited in celecoxib-treated mice. Celecoxib has been reported to reduce expression of P-selectin and ICAM-1 in experimental inflammatory models such as experimental colitis (Cuzzocrea et al., 2001, 2002). In our study, we observed that celecoxib suppressed expression of P-selectin and ICAM-1 in the brain of EAE mice. Since P-selectin and ICAM-1 are the adhesion molecules involved in the recruitment of inflammatory cells into CNS (Engelhardt et al., 1997; Dietrich, 2002; Scott et al., 2004), inhibition of cellular infiltration by celecoxib might be mediated by the downregulation of the expression of adhesion molecules.

Chemokines are also required for recruitment of immune cells into the CNS. MCP-1 is reported to be an essential chemokine in EAE (Hofmann et al., 2002). In the mouse model of atherosclerosis, Wang et al. (2005) reported that celecoxib decreased the inflammatory response and hyperplasia following vascular injury through inhibition of MCP-1 induction. We detected a decreased level of MCP-1 in the serum in celecoxib-treated mice on EAE. The suppression of MCP-1 by celecoxib might also contribute to the reduction of infiltrating cells into the CNS.

In conclusion, celecoxib has a potent therapeutic potential for EAE by inducing a Th2 bias and suppressing infiltration of inflammatory cells into the CNS through a COX-2-independent mechanism. Further analysis of celecoxib-mediated suppression of EAE will help drug development for multiple sclerosis. Celecoxib is hoped to be a new choice of the treatment of multiple sclerosis.

### Table 5 Serum level of MCP-1 in EAE mice after treatment with celecoxib

<table>
<thead>
<tr>
<th>EAE mice</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (CMC)</td>
<td>ND</td>
<td>60.0 ± 21.0</td>
<td>42.6 ± 17.0</td>
<td>ND</td>
</tr>
<tr>
<td>(n = 18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib (n = 16)</td>
<td>ND</td>
<td>8.5 ± 5.0*</td>
<td>12.9 ± 8.5</td>
<td>ND</td>
</tr>
<tr>
<td>Naive mice (n = 10)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

B6 mice were immunized with MOG35-55 peptide as described in Material and Methods. Serum samples from individual mice were collected on Day 0, 7, 10 and 14 after immunization. Serum concentration of MCP-1 was measured by ELISA. Data represent mean ± SEM (pg/ml). ND = not detectable. *P < 0.05 versus control.

### Acknowledgement

This study was supported by the Japan Research Foundation for Clinical Pharmacology.

### References


Miyamoto K, Oka N, Kawasaki T, Satoi H, Akiguchi I. The effect of celecoxib lacking the COX-2 inhibitor activity showed a novel mechanism independent of Erp44. They also demonstrated that an analogue of celecoxib lacking the COX-2 inhibitor activity showed identical effects to that of celecoxib on folding and secretion of IL-12, indicating that the effect is COX-2-independent. Since IL-12 is a key cytokine to provoke Th1 immune response, reduction in MOG-specific Th1 response is consistent with these previous findings.

The infiltration of immune cells in the CNS was significantly inhibited in celecoxib-treated mice. Celecoxib has been reported to reduce expression of P-selectin and ICAM-1 in experimental inflammatory models such as experimental colitis (Cuzzocrea et al., 2001, 2002). In our study, we observed that celecoxib suppressed expression of P-selectin and ICAM-1 in the brain of EAE mice. Since P-selectin and ICAM-1 are the adhesion molecules involved in the recruitment of inflammatory cells into CNS (Engelhardt et al., 1997; Dietrich, 2002; Scott et al., 2004), inhibition of cellular infiltration by celecoxib might be mediated by the downregulation of the expression of adhesion molecules.

Chemokines are also required for recruitment of immune cells into the CNS. MCP-1 is reported to be an essential chemokine in EAE (Hofmann et al., 2002). In the mouse model of atherosclerosis, Wang et al. (2005) reported that celecoxib decreased the inflammatory response and hyperplasia following vascular injury through inhibition of MCP-1 induction. We detected a decreased level of MCP-1 in the serum in celecoxib-treated mice on EAE. The suppression of MCP-1 by celecoxib might also contribute to the reduction of infiltrating cells into the CNS.

In conclusion, celecoxib has a potent therapeutic potential for EAE by inducing a Th2 bias and suppressing infiltration of inflammatory cells into the CNS through a COX-2-independent mechanism. Further analysis of celecoxib-mediated suppression of EAE will help drug development for multiple sclerosis. Celecoxib is hoped to be a new choice of the treatment of multiple sclerosis.


