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Effect of celecoxib, a cyclooxygenase-2 inhibitor, on the pathophysiology of adjuvant arthritis in rat

Masahiro Noguchi[a], Aishi Kimoto[a,*], Seiji Kobayashi[b], Taiji Yoshino[a], Keiji Miyata[a], Masao Sasamata[a]

[a]*Pharmacology Laboratories Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21, Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan*

[b]*Clinical Development Department, Drug Development Division, Yamanouchi Pharmaceutical Co., Ltd., 17-1, Hasune 3-chome, Itabashi-ku, Tokyo 174-8612, Japan*

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Abstract

We investigated the efficacy of celecoxib, a specific cyclooxygenase (COX)-2 inhibitor, on arthritic pathophysiology and confirmed its gastric safety in adjuvant-induced arthritis rats. Results were compared with those for loxoprofen, a non-selective COX inhibitor. Arthritis was induced by injection of 1 mg of *Mycobacterium butyricum* in 50 μl of liquid paraffin into the left footpad of Lewis rats. The drugs were given by twice daily oral administration for 10 days beginning 15 days after adjuvant injection, with celecoxib at 0.01–3 mg/kg/day and loxoprofen at 0.01–3 mg/kg/day. Celecoxib significantly inhibited paw swelling, hyperalgesic response, and joint destruction (radiographic and histopathological findings) in these arthritic rats. These effects of celecoxib were superior to those of loxoprofen. Further, the administration of loxoprofen (3 mg/kg/day) caused significant gastric lesions, whereas celecoxib at the same dose did not. These results suggest that COX-2-mediated prostaglandins may play an important role in the progression of pathophysiology in this model and that celecoxib may be a useful therapeutic agent for the treatment of rheumatoid arthritis, with greater safety than non-selective COX inhibitors.

Keywords: Adjuvant-induced arthritis; Cyclooxygenase-2; Non-steroidal anti-inflammatory drug; Celecoxib

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of conditions characterized by pain or inflammation such as rheumatoid arthritis and osteoarthritis, and it is well known that their therapeutic effect is due to the inhibition of cyclooxygenase (COX) (Vane, 1971). Two isoforms of COX have been identified, COX-1 and COX-2 (Smith and Dewitt, 1996; Vane et al., 1998). COX-1 is constitutively expressed in healthy tissues such as platelets, stomach, and kidneys, and generates prostaglandins which maintain homeostasis (O'Neill and Ford-Hutchinson, 1993; Simon, 1996). COX-2 is also expressed at a basal level in certain tissues such as brain and kidneys, but its expression is up-regulated by exposure to pro-inflammatory cytokines, and it plays a pivotal role in inflammation, pain, and fever (Katori and Majima, 2000; Seibert et al., 1994). Conventional NSAIDs inhibit both COX-1 and COX-2 at standard anti-inflammatory doses, and this dual inhibition may lead to a number of side effects, in particular gastrointestinal ulceration (Wallace et al., 2000; Tanaka et al., 2001).

Rheumatoid arthritis is characterized by chronic swelling and inflammation of the synovial membrane that lines joints. As rheumatoid arthritis progresses, the linings of joints degenerate, leading to severe pain and decreased joint mobility and significantly impacting on the quality of life. Rheumatoid synovial tissues express COX-2 (Kang et al., 1996), and its activation in rheumatoid synovial cells is

* Corresponding author. Tel.: +81 29 863 6639; fax: +81 29 854 1616.
E-mail address: kimoto@yamanouchi.co.jp (A. Kimoto).

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enhanced by pro-inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α (Angel et al., 1994; Szczepanski et al., 1994; Mino et al., 1998). COX-2-specific inhibitors are therefore expected to have anti-inflammatory and analgesic activities with a reduced risk of the gastrointestinal ulcerogenicity observed with NSAIDs.

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-pyrazol-1-yl] benzenesulfonamide, is a specific inhibitor of COX-2 (Penning et al., 1997). Here, we investigated the effects of celecoxib on arthritic pathologies (inflammation, pain, and joint destruction) and the incidence of gastric lesions in adjuvant-induced arthritis rats, and compared the results with those for loxoprofen, a non-specific COX inhibitor.

2. Materials and methods

2.1. Animals

Male Lewis rats (Charles River Japan, Kanagawa, Japan) weighing 195–240 g were used. All procedures used in this study complied with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

2.2. Drugs and reagents

Drugs used were celecoxib (Pfizer, Inc., NY, USA) and loxoprofen $Na \cdot 2H_2O$ (Shiono Chemical, Tokyo, Japan). Loxoprofen sodium was used as loxoprofen, with doses shown as the free form.

2.3. Induction of adjuvant arthritis

Adjuvant arthritis was induced by the injection of 1 mg of *Mycobacterium butyricum* desiccated (Difco Laboratories, Detroit, MI, USA) in 50 μl of paraffin oil into the left hind paw (Day 0). Celecoxib (0.1–3 mg/kg/day) and loxoprofen (0.1–3 mg/kg/day) were suspended in vehicle (0.5% methylcellulose and 0.025% Tween-20 solution) and given by twice daily oral administration from days 15 to 24 after adjuvant injection. Right contralateral hind paw volume was measured by water displacement using a plethysmometer (Muromachi Kikai, Tokyo, Japan) on day 25 after adjuvant injection. Pain threshold of the right hind paw was determined by the number of squeak vocalizations induced by five consecutive gentle flexions of the right ankle joint at 3-s intervals (Kuzuna and Kawai, 1975; Winter et al., 1979).

2.4. Biochemical measurement in plasma

On day 25 after adjuvant injection, the animals were dissected under anesthesia with ether. Whole blood was taken from the inferior vena cava with a heparin-added syringe and the animals were sacrificed by bleeding. Blood samples were centrifuged to isolate plasma, and plasma prostaglandin E_2 and α_1-acid glycoprotein levels in normal, vehicle-, celecoxib (3 mg/kg/day)-, and loxoprofen (3 mg/kg/day)-treated animals were measured by enzyme immunoassay (EIA) (Prostaglandin E_2 EIA kit; Cayman Chemical, MI, USA) (α_1-acid glycoprotein EIA kit; Panapharm Laboratories, Kumamoto, Japan).

2.5. Assessment of joint destruction (joint mobility, radiographic evaluation, and histopathological evaluation)

On day 25 after adjuvant injection, the right hind limb was sectioned below the hip joint and fixed in 10% formaldehyde. Joint mobility was defined as the extent of movement of the right ankle joint. Radiographs of the adjuvant non-injected hind limb were taken with a Softex-CMB X-ray unit (Softex, Kanagawa, Japan). The severity of bone damage on the radiographs was assessed blindly by the grading of periostitis and bone destruction. Histopathological evaluation of joints was made by the assessment of neutrophil infiltration, bone destruction, osteoclast proliferation, and osteogenesis. Radiographic and histopathological evaluations were scored as 0, negative; 1, minimal; 2, mild; 3, moderate; 4, severe; and 5, catastrophic, and the scores of each histological parameter were summed for each animal.

2.6. Assessment of gastric lesions

After sacrifice, the stomach were excised, opened by cutting along the greater curvature, and observed macroscopically for gastric mucosal lesions. The incidence of lesions was calculated from the number of animals with gastric lesions per group ($n=10$).

2.7. Statistical analysis

Data are presented as the mean ± S.E.M of 10 animals. ED_{50} values and their 95% confidence intervals (95% CI) were calculated by linear regression analysis. The significance of differences between the normal and vehicle-treated adjuvant-induced arthritis groups was determined by Student's *t*-test, and between the vehicle-treated and drug-treated groups by Dunnett's multiple range test. The significance of differences in gastric ulcerogenicity (the number of animals with one or more lesions per number of animals tested) was determined by the χ^2 test and revised by Bonferroni correction for multiple comparisons. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of celecoxib and loxoprofen on hind paw swelling in adjuvant-induced arthritis rats

Celecoxib and loxoprofen decreased paw volume in a dose-dependent manner in adjuvant-induced arthritis rats.

Table 1
The effects of celecoxib and loxoprofen on hind paw swelling in adjuvant-induced arthritis rats

Drug	ED_{50} (mg/kg/day) (95% CI)	Maximum inhibition (%)
Celecoxib	0.4 (0.2–0.7)	84.2
Loxoprofen	1.7 (1.2–2.8)	62.8

Celecoxib (0.1–3 mg/kg/day) and loxoprofen (0.1–3 mg/kg/day) were given by twice daily oral administration from days 15 to 24 after adjuvant injection. ED_{50} values and their 95% confidential interval (95% CI) were determined by linear regression analysis.

Maximum inhibitory rates in the drug-treated groups were 84.2% (celecoxib) and 62.8% (loxoprofen). The ED_{50} values for celecoxib and loxoprofen were 0.4 and 1.7 mg/kg/day, respectively (Table 1, Fig. 1).

3.2. Analgesic effects of celecoxib and loxoprofen in adjuvant-induced arthritis rats

Vocalization frequency was significantly increased in the vehicle-treated adjuvant-induced arthritis group compared with the normal group. Celecoxib (1 and 3 mg/kg/day) and loxoprofen (3 mg/kg/day) significantly decreased vocalization frequency compared with the vehicle-treated adjuvant-induced arthritis group (Fig. 2).

3.3. Effects of celecoxib and loxoprofen on inflammatory parameters in adjuvant-induced arthritis rats

Plasma prostaglandin E_2 and α_1-acid glycoprotein, which are parameters of inflammation, were significantly increased by the injection of adjuvant. Therapeutic administration of celecoxib (3 mg/kg/day) and loxoprofen (3 mg/kg/day) inhibited the increases in these parameters (Fig. 3).

3.4. Effects of celecoxib and loxoprofen on joint destruction in adjuvant-induced arthritis rats

In the vehicle-treated adjuvant-induced arthritis group, joint mobility was significantly decreased compared with the normal group. Celecoxib (1 and 3 mg/kg/day) and loxoprofen (3 mg/kg/day) significantly improved joint mobility as compared with the vehicle-treated adjuvant-induced arthritis group (Fig. 4).

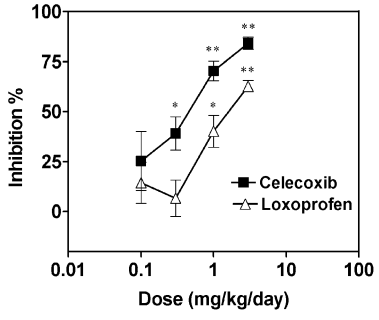

Fig. 1. Inhibitory effects of celecoxib and loxoprofen on hind paw swelling in adjuvant-induced arthritis rats. Each point represents the mean ± S.E.M. of the percentage inhibition of mean of vehicle-treated values ($n=10$). *$P<0.05$, **$P<0.01$ vs. vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

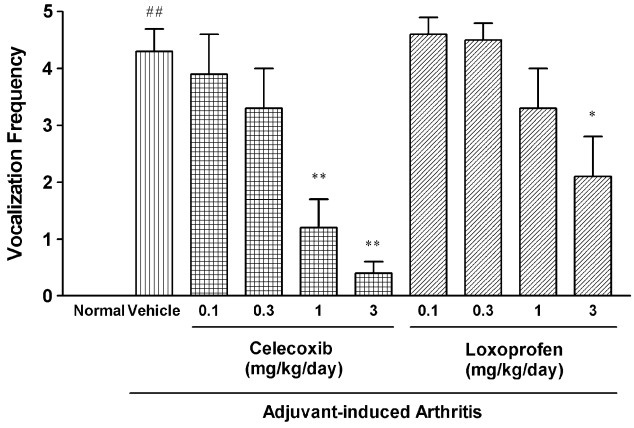

Fig. 2. Effects of celecoxib, loxoprofen, and indomethacin on joint flexion-induced pain in adjuvant-induced arthritis rats. Each column represents the mean ± S.E.M. of vocalization frequency ($n=10$). ##$P<0.01$ vs. normal group (Student's t-test). *$P<0.05$, **$P<0.01$ vs. vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

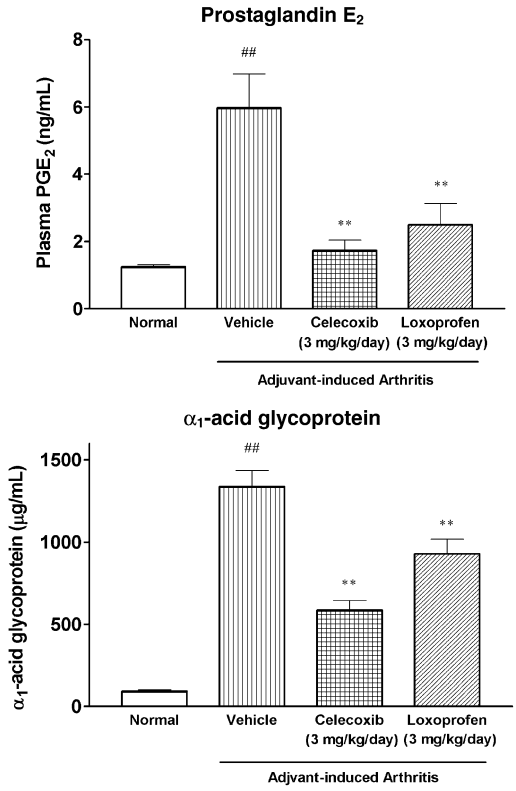

Fig. 3. Effects of celecoxib and loxoprofen on plasma inflammatory parameters (plasma prostaglandin E_2 and α_1-acid glycoprotein) in adjuvant-induced arthritis rats. Each drug was given by twice daily oral administration from days 15 to 24 after adjuvant injection. Each column represents the mean ± S.E.M. ($n=10$). ##$P<0.01$ vs. normal group (Student's t-test). **$P<0.01$ vs. the vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

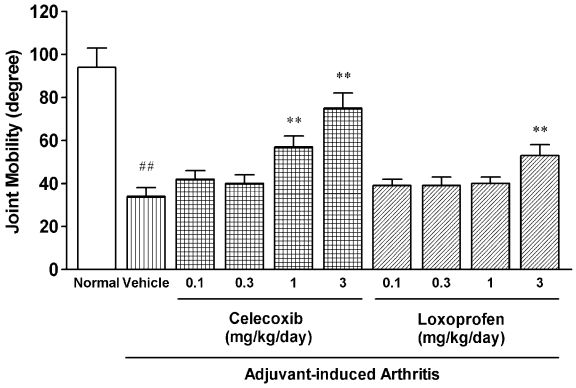

Fig. 4. Effects of celecoxib and loxoprofen on joint mobility in adjuvant-induced arthritis rats. The degree of flexion and extension of the right ankle joint was measured, and the difference expressed as joint mobility. Each column represents the mean ± S.E.M. of joint mobile angle ($n=10$). $^{\#\#}P<0.01$ vs. normal group (Student's t-test) $^{**}P<0.01$ vs. the vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

As shown in Figs. 5 and 6, radiography of the hind limb from the vehicle-treated adjuvant-induced arthritis group showed severe disorganization (periostitis and destruction) of bone in the distal tibia, tarsus, metatarsus, and calcaneus (Fig. 5B). Celecoxib (3 mg/kg/day) clearly ameliorated this periostitis and bone destruction (Fig. 5C), whereas loxoprofen showed no obvious effect (Fig. 5D).

In histopathological evaluation, no animal was graded as catastrophic in any group. In the bone, celecoxib (1 and 3 mg/kg/day) and loxoprofen (3 mg/kg/day) significantly

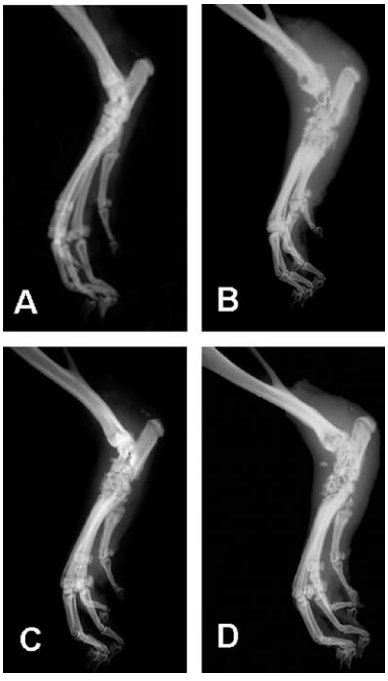

Fig. 5. Effects of celecoxib and loxoprofen on joint destruction in adjuvant-induced arthritis rats (X-ray images). Radiographs were taken from the adjuvant non-injected hind paw on day 25 after adjuvant injection. Normal (A), vehicle (B), celecoxib (3 mg/kg/day) (C), and loxoprofen (3 mg/kg/day) (D).

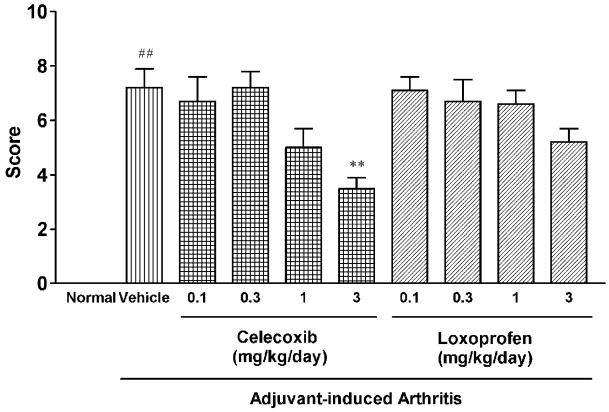

Fig. 6. Effects of celecoxib and loxoprofen on joint destruction in adjuvant-induced arthritis rats (radiographic evaluation). Each column represents the mean ± S.E.M. of the sum of scores (periostitis and bone destruction) of intertarsal joint destruction by X-ray estimation using a six-grade score in each animal ($n=10$). $^{\#\#}P<0.01$ vs. normal group (Student's t-test). $^{**}P<0.01$ vs. vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

reversed the bone destruction associated with arthritis (Figs. 7 and 8).

3.5. Gastric ulcerogenicity of celecoxib and loxoprofen in adjuvant-induced arthritis rats

As shown in Table 2, the vehicle-treated adjuvant-induced arthritis group showed a tendency to an increase in gastric lesions compared with the normal rats. Administration of loxoprofen produced severe gastric lesions in more than half of the animals. No differences were seen between vehicle-treated adjuvant-induced arthritis and celecoxib groups.

4. Discussion

Limb swelling, inflammatory cell infiltration, proliferative synovitis, and erosion of the bone and cartilage structure are clinical findings common to human arthritis and adjuvant-induced arthritis rat (Pearson and Wood, 1959). Owing to this similarity in pathologic features, the adjuvant-induced arthritis rat is a widely used model of rheumatoid arthritis in evaluating the efficacy of anti-inflammatory drugs (Pearson, 1956; Pearson and Wood, 1959). Further, COX-2 protein expression increases at inflammatory sites with the development of arthritis in both animals and humans (Kang et al., 1996; Anderson et al., 1996).

In the present study, we investigated the anti-arthritic activities of celecoxib, a specific COX-2 inhibitor, in adjuvant-induced arthritis rats, in comparison with those of a non-selective COX inhibitor. Results showed that celecoxib significantly inhibited paw swelling in these rats, and that this inhibition was accompanied by a decrease in plasma prostaglandin E_2 level. In previous reports, inflam-

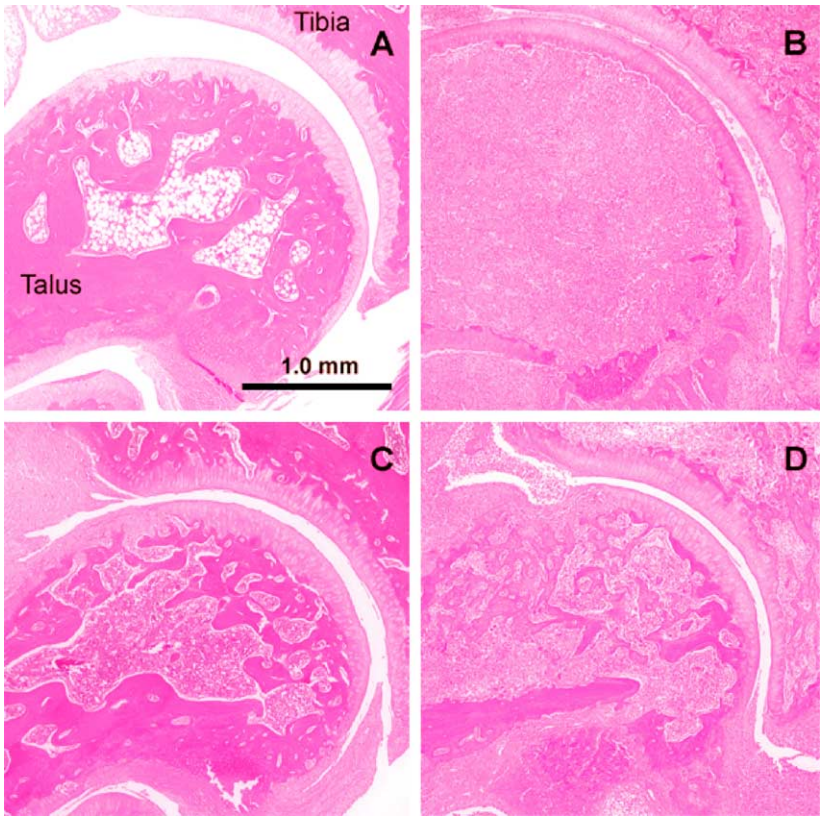

Fig. 7. Effects of celecoxib and loxoprofen on joint destruction in adjuvant-induced arthritis rats (tibia–talus joint histological image). Histological images were taken from the adjuvant non-injected hindlimb on day 25 after adjuvant injection. Sections were stained with hematoxylin and eosin. Normal (A), vehicle (B), celecoxib (3 mg/kg/day) (C), and loxoprofen (3 mg/kg/day) (D). Bar indicates 1.0 mm.

mation and plasma prostaglandin E_2 levels were increased after adjuvant injection (Anderson et al., 1996; Melli, 1988). The decrease in plasma prostaglandin E_2 level by celecoxib was also concomitant with a decrease in the plasma level of α $_1$-acid glycoprotein, an acute phase protein whose serum levels are reported to reflect systemic disease activity in rheumatoid arthritis (Nakamura et al., 1993). These findings indicate that the COX-2 inhibition by celecoxib not only

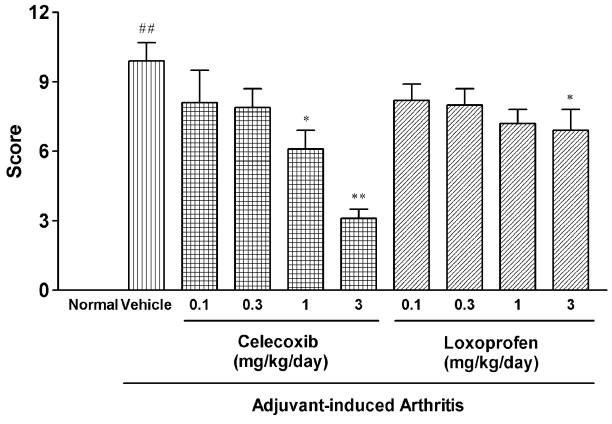

Fig. 8. Effects of celecoxib and loxoprofen on joint destruction in adjuvant-induced arthritis rats (histopathological evaluation). Histological images were taken from the adjuvant non-injected hindlimb on day 25 after adjuvant injection. Each column represents the mean ± S.E.M of the sum of scores of bone (infiltration of neutrophils, destruction of bone tissue, increase in osteoclasts, and growth of new bone). $^{\#\#}P<0.01$ vs. normal group (Student's t-test). $^{*}P<0.05$, $^{**}P<0.01$ vs. vehicle-treated adjuvant-induced arthritis group (Dunnett's multiple range test).

Table 2
Gastric ulcerogenicity of celecoxib and loxoprofen in adjuvant-induced arthritis rats

Drugs	Doses (mg/kg/day)	Number of animals with gastric lesion/total animals used
Normal		0/10
Vehicle		3/10
Celecoxib	0.1	3/10
	0.3	4/10
	1	5/10
	3	3/10
Loxoprofen	0.1	6/10
	0.3	5/10
	1	7/10
	3	10/10[a]

Drugs were given by twice daily oral administration from days 15 to 24 after adjuvant injection. On day 25 after adjuvant injection, animals were sacrificed and the incidence of gastric lesions was calculated. Each value represents the number of animals with gastric lesion per the number of tested animals ($n=10$).

[a] $P<0.005$ vs. the vehicle-treated adjuvant-induced arthritis group (χ^2-test significance level is revised by Bonferroni correction for multiple comparison).

decreases inflammatory prostaglandin E_2, but also leads to a remission in the systemic progression of rheumatoid arthritis.

Joint inflammation causes pain and stiffness, the major symptomatic complaints in rheumatoid arthritis. Under these conditions, mechanical and physical stimulation can cause severe pain. In order to investigate the analgesic activity of celecoxib in adjuvant-induced arthritis rats, flexion test was used. Flexion test seems to be a good correlation of potency in this model with clinical potency (Pircio et al., 1975). In our study, joint mobility in these adjuvant-induced arthritis rats was significantly restricted, and the joints were hypersensitive to light flexion. Against this, however, celecoxib improved joint mobility and showed good analgesic activity. These effects were investigated in greater detail by radiographic and histopathological evaluation of the hindlimbs of arthritic animals. Results showed that the therapeutic administration of celecoxib was significantly protective against periostitis and bone destruction. In the bone destruction of rheumatoid arthritis, COX-2-derived prostaglandin E_2 from osteoblasts is produced by various stimuli such as mechanical force, local hormones, and cytokines, and stimulates osteoclast formation (Ryder and Duncan, 2000; Maciel et al., 1997; Xu et al., 1997; Akatsu et al., 1991; Sato et al., 1996; Tai et al., 1997). Igarashi et al. (2002) reported that celecoxib inhibited the osteoclast formation induced by interleukin-1β, tumor necrosis factor-α, and lypopolysaccharide in vitro. These reports and our present data suggest that COX-2-derived prostaglandin E_2 plays an important role in the bone destruction of rheumatoid arthritis, and that celecoxib has beneficial effects on disease progression and symptoms in arthritis patients.

The present results showed that the anti-inflammatory effects of celecoxib were more potent than those of loxoprofen. This difference in anti-arthritic activity may be based on differences in their inhibitory potency for COX-2. A previous study showed that the inhibitory potency of celecoxib for COX-2 was equal or superior to that of indomethacin in recombinant human COX-2 assay (Gierse et al., 1999). Another reported that the inhibitory potency of loxoprofen-SRS, an active metabolite of loxoprofen sodium, for COX-2 was weaker than that of indomethacin in human interleukin-1β-stimulated synovial cells (Kawai et al., 1998). In addition, Kusunoki et al. (2002) demonstrated that celecoxib, but not other COX-2 inhibitors, induced apoptosis in rheumatoid arthritis synovial fibroblasts independent of COX-2 inhibition. It is therefore conceivable that the difference between celecoxib and loxoprofen in their effect on the course of arthritic changes in adjuvant-induced arthritis might reflect a difference in their inhibitory effect against COX-2, and on the apoptotic action of celecoxib.

The major side effect of conventional NSAIDs is their induction of gastrointestinal lesions. These are thought to result from the dual inhibition of COX-1 and COX-2 in gastrointestinal tissues (Wallace et al., 2000; Tanaka et al., 2001). Interestingly, arthritic patients are at greater risk of developing NSAID-induced ulcers than non-arthritic NSAIDs users (Fries et al., 1989). Moreover, adjuvant-induced arthritic rats are highly sensitive to the gastric damaging activity of these drugs (McCafferty et al., 1995; Schleyerbach and Wedde, 1984). In the present study, gastric lesions were also observed in vehicle-treated arthritic animals, but celecoxib produced no further aggravation of these lesions. In contrast, loxoprofen caused gastric lesions in more than half of all animals at 3 mg/kg/day. Taken together, these findings demonstrate that celecoxib has a wider safety index than loxoprofen.

In conclusion, this study shows that COX-2 plays an important role in the progression of rheumatoid arthritis pathology, and that COX-2 inhibitors are likely to be effective agents in the treatment of this condition with a low risk of the gastrointestinal damage observed in the usage of NSAIDs.

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