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

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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

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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 $\alpha_1$-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) ($\alpha_1$-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 $\chi^2$ 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.