The Anti-inflammatory Effect of FR188582, a Highly Selective Inhibitor of Cyclooxygenase-2, With an Ulcerogenic Sparing Effect in Rats

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ABSTRACT—The anti-inflammatory and ulcerogenic effects of FR188582, 3-chloro-5-[4-(methylsulfonyl)phenyl]-1-phenyl-1H-pyrazole, were investigated. In a recombinant human cyclooxygenase (COX) enzyme activity, FR188582 inhibited COX-2 with an IC50 value of 0.017 μM, and the inhibition of prostaglandin (PG) E2 formation by FR188582 was over 6000 times more selective for COX-2 than COX-1. Oral administration of FR188582 dose-dependently inhibited adjuvant arthritis. This effect was threefold more potent than that of indomethacin. FR188582 and indomethacin dose-dependently suppressed the formation of immunoreactive PGE2, but not immunoreactive leukotriene (LT) B4, in arthritic paw. Unlike indomethacin, FR188582 did not induce visible gastric lesions in rats at doses up to 32 mg/kg, p.o. Furthermore, FR188582 did not inhibit the level of immunoreactive PGE2 and immunoreactive 6-keto PGF1α in rat gastric mucosa. These results suggest that FR188582, a highly selective COX-2 inhibitor, has a potent anti-inflammatory effect mediated by inhibition of PGE2 in inflamed tissues. The safety profile of FR188582 appears to be improved over the safety profile of indomethacin.

Keywords: FR188582, Cyclooxygenase-2, Adjuvant arthritis, PGE2 formation, Gastric lesion

Cyclooxygenase (COX) is a key enzyme in the formation of prostaglandins (PGs), which are important mediators of inflammation (1). Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin and aspirin, are widely used to treat chronic inflammatory states such as rheumatoid arthritis and osteoarthritis, and it is well known that their effect is due to inhibition of COX (2). However, side effects such as gastrointestinal irritation and renal function abnormalities after long-term administration with NSAIDs have arisen in clinical use (3, 4).

In recent years, two isoforms of COX have been identified (5, 6). The biochemical profiles of housekeeping COX-1 and inducible COX-2 are distinctly different and, in general, COX-1, which is constitutively expressed in the stomach, sustains the routine physiologic function of PGs, including gastric mucosal protection; whereas COX-2 is chiefly induced by exposure of certain cytokines, mitogens and endotoxin and is found in sites of inflammation (5, 7, 8). Therefore, agents that specifically inhibit COX-2 may produce an anti-inflammatory activity with a reduced risk of ulcerogenic toxicity.

Recently, FR188582 (3-chloro-5-[4-(methylsulfonyl)phenyl]-1-phenyl-1H-pyrazole), the chemical structure of which is shown in Fig. 1, has been synthesized as a novel selective inhibitor of COX-2. In this study, we investigated the pharmacological profile focusing on the anti-inflammatory and ulcerogenic effects of FR188582. The findings were compared with indomethacin.

MATERIALS AND METHODS

Animals

Ethical guidelines for the experimental use of animals were followed (9). In addition, the experimental work was reviewed by the Fujisawa Pharmaceutical Animal Experiment Committee for Animal Experimentation.

Female Lewis rats (140 – 180 g; Charles River Japan, Yokohama) at the age of 8 weeks were used. The animals were maintained in a group of 5 animals on a 12-h light-dark cycle (light on from 0700 to 1900 h) in a controlled temperature (23 ± 1°C) and humidity (55 ± 5%) environment. The rats were given standard laboratory food and tap water ad libitum.
Drugs

Indomethacin was obtained from Sigma (St. Louis, MO, USA). FR188582 was chemically synthesized at Fujisawa Pharmaceutical (Osaka).

**Human recombinant COX-1 and COX-2 enzyme activity**

Human recombinant COX-1 and COX-2 were expressed in Chinese hamster ovary cells. The appropriate COX enzyme (1 μg for COX-1 and/or 3 μg for COX-2) was pre-incubated in 100 mM Tris-HCl buffer (pH 7.3) containing hematin (2 μM) and tryptophan (5 mM) with drugs (0.0001 – 100 μM) dissolved in 1% dimethylsulfoxide for 5 min at 37°C prior to the addition of arachidonic acid (10 μM) for 5 min at 37°C. Reactions were terminated by the addition of 1 N HCl, and PGE$_2$ production was measured by radioimmunoassay (Amersham, Buckinghamshire, England).

**Induction of adjuvant arthritis**

Adjuvant arthritis was induced in female Lewis rats by intradermal injection into the planter surface of the right hind paw of 0.5 mg of a suspension of heat-killed and dried *Mycobacterium tuberculosis* H37 RA (Difco, Detroit, MI, USA) in 0.05 ml of liquid Paraffin (day 0) (10, 11). The technique of Opas et al. was used (12). Rats were euthanized by CO$_2$ inhalation 24 days after adjuvant injection. The stomach was rapidly excised, washed, chopped into pieces with a diameter of 4 mm and incubated with distilled water to a final concentration of 15% methanol. The stomach was opened by cutting along the greater curvature, and the index was assessed by scoring zero to four gastric lesions. Petechiae were assigned a score of 1, and erosion was assigned a score of 2. The gastric mucosal lesions were scored according to their number (a score of 3 for one to four lesions and a score of 4 for five or more lesions).

**Determination of PGs contents in the gastric mucosa**

The technique of Whittle et al. was used (15). Adjuvant-injected rats were treated once a day with drugs from day 15 to day 24 after adjuvant injection. Rats were euthanized by CO$_2$ inhalation 5 h after the last administration of drugs on day 24. The stomach was rapidly excised, washed, chopped into pieces with a diameter of 4 mm and incubated in 20 mM Tris-Hank’s HCl buffer (pH 7.4, 1 ml) by vortex mixing for 1 min at room temperature. After centrifugation at 3,000 rpm for 10 min at 4°C, the supernatant was assayed for PGE$_2$, LTB$_4$, and 6-keto PGF$_{1α}$ by radioimmunoassay (Amersham, Buckinghamshire, England).

**Statistical analyses**

Obtained results are expressed as the mean ± S.E.M. Statistical significance was analyzed using the one-way analysis significance (ANOVA) followed by Dunnett’s multiple comparison test. ED$_{50}$ value and their 95% confi-
RESULTS

Effects of FR188582 on human recombinant COX-1 and COX-2 enzymes in vitro

Figure 2 shows the effects of FR188582 on the activity of recombinant human COX-1 and COX-2. FR188582 inhibited COX-2 enzyme in a concentration-dependent manner with an IC₅₀ value of 0.017 ± 0.0085 µM. In contrast, FR188582 (at concentrations of 0.1 – 100 µM) only weakly inhibited COX-1 (only 46% at 100 µM). FR188582 showed a high selectivity for COX-2, over 6000 times relative to COX-1. The IC₅₀ values of the reference compound indomethacin for COX-1 and COX-2 were 0.10 ± 0.074 and 0.26 ± 0.095 µM, respectively.

Anti-inflammatory effect of FR188582 in adjuvant arthritic rats

As shown in Fig. 3, oral administration of FR188582 (0.01 – 3.2 mg/kg) reversed paw edema in adjuvant arthritic rats and showed a therapeutic effect in a dose-dependent manner with ED₅₀ values (95% C.L.) of 0.074 (0.00021 – 0.53) and 0.063 (0.0039 – 0.31) mg/kg for adjuvant-injected paws and adjuvant-uninjected paws, respectively. The anti-inflammatory effect of FR188582 was threefold more potent than that of indomethacin with ED₅₀ values (95% C.L.) of 0.24 (0.047 – 1.8) and 0.20 (0.021 – 0.79) mg/kg for adjuvant-injected paws and adjuvant-uninjected paws, respectively (Fig. 4).

Effects of FR188582 on the formation of arachidonic acid metabolites (PGE₂ and LTB₄) in adjuvant arthritic rat paws

Oral administration of FR188582 (0.01 – 3.2 mg/kg) dose-dependently reduced the formation of immunoreactive PGE₂ in arthritic paws with ED₅₀ values (95% C.L.) of 0.11 (0.0070 – 0.61) and 0.068 (0.0078 – 0.24) mg/kg for adjuvant-injected paws and adjuvant-uninjected paws, respectively (Figs. 5 and 6). However, therapeutic treatment with FR188582 at doses up to 3.2 mg/kg (p.o.) did not affect the formation of immunoreactive LTB₄ in either of the arthritic paws. Indomethacin also showed a dose-dependent inhibition of the formation of immunoreactive PGE₂ with ED₅₀ values (95% C.L.) of 0.20 (0.025 – 1.9) and 0.13 (0.022 – 0.60) mg/kg for adjuvant-injected paws and adjuvant-uninjected paws, respectively, but not immunoreactive LTB₄, in the arthritic rat paws.

Gastric tolerability of drugs in rats

Drugs were administered orally once a day from day 15 to day 24 after adjuvant injection. Therapeutic treatment in adjuvant arthritis with FR188582 at doses between 0.32 and 32 mg/kg did not induce any mucosal lesions (Table 1). In contrast, indomethacin at a dose of 3.2 mg/kg induced marked gastric lesions in two of five rats. In the rats administered indomethacin at 10 mg/kg, one of five rats died on day 17, three of five rats died on day 18 and one of five rats died on day 19.
Effects of FR188582 on the contents of PGE$_2$ and 6-keto PGF$_{1α}$ in rat gastric mucosa ex vivo

Therapeutic treatment in adjuvant arthritis with FR188582 at doses between 0.01 and 32 mg/kg did not inhibit the level of immunoreactive PGE$_2$ and immunoreactive 6-keto PGF$_{1α}$ in rat gastric mucosa (Fig. 7). On the other hand, indomethacin inhibited the level of immunoreactive PGE$_2$ and immunoreactive 6-keto PGF$_{1α}$ in rat gastric mucosa with ED$_{50}$ values (95% C.L.) of 0.11 (0.023–0.48) and 0.24 (0.070–2.3) mg/kg, respectively. The arachidonic acid metabolite immunoreactive LTB$_4$ was not detected in the normal group and in the adjuvant-control group (data not shown). The sensitivity of the LTB$_4$ assay was 0.062 ng/section.
DISCUSSION

The present study demonstrates that FR188582 is a highly selective inhibitor of COX-2 in vitro and shows an anti-inflammatory effect without gastric mucosal damage. The mechanism of inhibition of COX activity by FR188582 is very similar to those reported with other well-known selective COX-2 inhibitors, NS-398, celecoxib and refecoxib (17–19).

In vitro, FR188582 caused only weak inhibition of PGE$_2$. 

Fig. 5. Effects of FR188582 on the formation of arachidonic acid metabolites in adjuvant injected right arthritic rat paws. Drugs were given orally once a day therapeutically from day 15 to day 24 after adjuvant injection. Rats were euthanized by CO$_2$ inhalation 24 days after immunization, and arachidonic acid metabolites, PGE$_2$ (A) and LTB$_4$ (B), in inflamed rat right hind paws were extracted and analyzed by radioimmunoassay. Significantly different from the adjuvant-control, **P<0.01, *P<0.05, ***P<0.01. Values were corrected for recovery efficiency and expressed as ng/paw ± S.E.M., n = 5.

Fig. 6. Effects of FR188582 on the formation of arachidonic acid metabolites in adjuvant uninjected left arthritic rat paws. Drugs were given orally once a day therapeutically from day 15 to day 24 after adjuvant injection. Rats were euthanized by CO$_2$ inhalation 24 days after immunization, and arachidonic acid metabolites, PGE$_2$ (A) and LTB$_4$ (B), in inflamed rat left hind paws were extracted and analyzed by radioimmunoassay. Significantly different from the adjuvant-control, **P<0.01, ***P<0.01. Values were corrected for recovery efficiency and expressed as ng/paw ± S.E.M., n = 5.
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synthesis (IC50 >100 μM) in recombinant human COX-1, whereas the much lower IC50 value of 0.017 μM was obtained in recombinant human COX-2. In contrast, the classical NSAID indomethacin inhibited both COX-1 and COX-2 activity to a similar degree with IC50 values of 0.10 and 0.26 μM, respectively; thus it can be considered as a non-selective COX inhibitor. These results indicate that FR188582 has a high selectivity for the inhibition of COX-2 over COX-1, unlike indomethacin.

Next, we evaluated the anti-inflammatory effect of FR188582 using the established rat adjuvant arthritis assay, which is an animal model of chronic inflammation resembling the clinical manifestations of rheumatoid arthritis. The primary swelling of adjuvant-injected hind paws is observed between day 1 and day 4 after mycobacterial adjuvant injection, and the secondary swelling, which is characterized by inflammation of both the paws and legs, especially the adjuvant uninjected hind paws, occurs with a delay of approximately 10 days after adjuvant injection (11). Therapeutic treatment with FR188582 showed an anti-inflammatory effect for the secondary swelling in adjuvant arthritic rat paws in a dose-dependent manner similarly to indomethacin. A similar observation has been reported with other selective COX-2 inhibitors, NS-398, celecoxib and refecoxib, in this model (20–22). PGE2 production is associated with upregulation of COX-2 mRNA and protein in rat adjuvant arthritic paws (23, 24). These results suggest that COX-2 plays a role in rat adjuvant arthritis. To evaluate the relationship between the edema and the production of PG in the inflamed rat paws induced by adjuvant injection, we extracted arachidonate acid metabolites, especially PGE2 and LTB4, from rat adjuvant arthritic paws after treatment with drugs, FR188582 and indomethacin. Oral administration of FR188582, a highly selective COX-2 inhibitor, dose dependently inhibited the

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**Table 1.** Gastric ulcerogenicity of the drugs in adjuvant-induced arthritic rats

<table>
<thead>
<tr>
<th>Drug (mg/kg, p.o.)</th>
<th>Ulcer index</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Adjuvant-control</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>FR188582 0.32</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3.2</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>32</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Indomethacin 0.32</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3.2</td>
<td>0.8 ± 0.5</td>
<td>40.0</td>
</tr>
<tr>
<td>10 n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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</table>

Drugs were administered orally once a day from day 15 to day 24 in adjuvant-treated rats. On day 24, visible gastric lesions were scored (score scales: petechiae = 1, erosion = 2, lesions between one and four = 3, lesions greater than five = 4). Values are the mean ± S.E.M., n = 5. n.d., No data because all the rats died.
formation of PGE\(_2\) but not LTB\(_4\) in both inflamed rat paws similarly to indomethacin. The ED\(_{50}\) values of FR188582 for the inhibitory effect on PGE\(_2\) production in both inflamed paws were almost the same as the ED\(_{50}\) values of FR188582 for its anti-inflammatory effect in adjuvant arthritis. Our result also confirms the observation reported by Anderson et al. (24) indicating that the development of adjuvant arthritis is associated with the upregulation of PGE\(_2\) produced exclusively by COX-2. Therefore, this model provides a direct in vivo biochemical measurement for COX-2 activity in the inflammatory site.

While the inhibitory effect of FR188582 on recombinant human COX-2 was 15 times more potent than that of indomethacin, the anti-inflammatory effect of FR188582 in adjuvant arthritis model was only three-fold more potent than that of indomethacin. This discrepancy on the comparative effects of FR188582 and indomethacin in vitro and in vivo models suggests that indomethacin may give much higher blood and tissue levels than FR188582. The anti-inflammatory effect of FR188582 is more potent than that of other well-known selective COX-2 inhibitors, NS-398, celecoxib and refecoxib, with ED\(_{50}\) values of 4.69, 0.37 and 0.7 mg/kg, respectively (20 – 22). FK3311 is a selective inhibitor of COX-2 in inflamed tissues (25, 26). FK3311 shows an evident anti-inflammatory effect in adjuvant arthritic rat paws with ED\(_{50}\) values of 4.2 and 3.2 mg/kg for adjuvant-injected paws and adjuvant-uninjected paws, respectively (27). Thus, the anti-inflammatory effect of FR188582 in adjuvant arthritis was about 50 times more potent that of FK3311. These findings support the expectation that FR188582 is highly useful for the treatment of chronic inflammatory diseases such as rheumatoid arthritis.

The major side effect of conventional NSAIDs is the induction of gastrointestinal lesions, thought to be caused by the inhibition of COX-1 in the gastrointestinal tissues. In the present study, oral administration of indomethacin, which inhibits both COX-1 and COX-2 with approximately equal potency, caused a strong and dose-dependent gastric mucosal damage at low doses, whereas oral administration of the selective COX-2 inhibitor FR188582 at 400 times the ED\(_{50}\) for edema (32 mg/kg) resulted in no visible gastric mucosal lesions. The major difference between selective COX-2 inhibitors and non-selective NSAIDs is that selective COX-2 inhibitors have a good separation between their functional efficacy and ulcerogenic potentials, while non-selective NSAIDs have a narrow therapeutic index (20, 28, 29). COX-1 is the major COX isozyme present in the gastrointestinal tract (30). To investigate the relationship between the gastric damage and inhibition of COX-1 in the gastric mucosa following oral administration of NSAIDs, we measured ex vivo production of PGE\(_2\) and 6-keto PGF\(_{1\alpha}\), which are generated in the rat gastric mucosa. As expected, oral therapeutic treatment of indomethacin inhibited the contents of PGE\(_2\) and 6-keto PGF\(_{1\alpha}\) in rat gastric mucosa at a low dose (ED\(_{50}\) = 0.11 and 0.24 mg/kg, respectively), whereas oral administration of the highly selective COX-2 inhibitor FR188582 did not inhibit them at doses up to 32 mg/kg. Other selective COX-2 inhibitors, NS-398 and MK-966 (refecoxib), also fail to inhibit PG synthesis in the stomach (31, 32). The ED\(_{50}\) values of indomethacin for inhibition of PGE\(_2\) and 6-keto PGF\(_{1\alpha}\) contents in the rat gastric mucosa were nearly in the same range as the ED\(_{50}\) values of indomethacin for inhibition of PGE\(_2\) formation and edema in adjuvant arthritis. Indomethacin inhibited the production of both inflammatory site PGs and stomach PGs at similar doses. In this study, the selective COX-2 inhibitor FR188582 completely suppressed the production of PGs in the inflammatory site without reducing gastric PGs level. These observations strongly suggest that the strong ulcerogenic effect of indomethacin might be the result of its inhibition of stomach PGs produced by COX-1, but not COX-2.

In conclusion, we have shown that FR188582 is a highly selective COX-2 inhibitor that has a superior efficacy profile, compared with the classical NSAID indomethacin, in adjuvant arthritis. The pharmacology and safety profile of FR188582 is similar to that of recently described selective COX-2 inhibitors, NS-398, celecoxib and refecoxib. FR188582 may be clinically useful in the treatment of rheumatoid arthritis with dramatically improved margins of safety.

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