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Differential effect of FR122047, a selective cyclo-oxygenase-1 inhibitor, in rat chronic models of arthritis

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1 We investigated the effects of FR122047 (1-[(4,5-bis(4-methoxyphenyl)-2-thiazoyl)carbonyl]-4-methylpiperazine hydrochloride), a selective cyclo-oxygenase (COX)-1 inhibitor, in rat type II collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA).

2 Using an *ex vivo* rat whole blood assay, FR122047 (0.032–3.2 mg kg^{-1}) inhibited COX-1-derived thromboxane (TX) B_2 production with ED_{50} value of 0.059 mg kg^{-1}, indicating that it was orally active, but did not inhibit lipopolysaccharide-induced prostaglandin (PG) E_2 production derived by COX-2.

3 Oral administration of FR122047 showed a dose-dependent anti-inflammatory effect in rat CIA with ED_{50} value of 0.56 mg kg^{-1}. This drug also dose dependently suppressed the levels of PGE_2 and TXB_2 in CIA rat paws with ED_{50} values of 0.24 and 0.13 mg kg^{-1}, respectively.

4 FR122047 had no effect in rat AIA model. In contrast, indomethacin, a non-selective COX inhibitor, was anti-inflammatory and reduced the formation of PGs in AIA rat paws.

5 Unlike indomethacin, chronic treatment of FR122047 did not damage the stomach mucosa in CIA rats.

6 These results demonstrate that COX-1 contributes to the oedema and the formation of PGE_2 and TXB_2 in rat CIA model, but not in rat AIA model.

7 We conclude that FR122047 has an orally active and anti-inflammatory effect mediated by inhibition of PGE_2 and TXB_2 produced by COX-1 at a site of inflammation induced by type II collagen and it may be a useful tool for studying the involvement of COX-1 in various *in vivo* models of inflammation.

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Keywords: FR122047; cyclo-oxygenase-1; anti-inflammation; type II collagen arthritis; prostaglandin formation

Abbreviations: AIA, adjuvant-induced arthritis; CIA, type II collagen-induced arthritis; CII, type II collagen; COX, cyclo-oxygenase; LPS, lipopolysaccharide; NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; TX, thromboxane

Introduction

It is well known that adjuvant-induced arthritis (AIA) and type II collagen-induced arthritis (CIA) are animal models of rheumatoid arthritis for detecting new anti-rheumatic drugs (Bartlett & Schleyerbach, 1985; Takeoka *et al*., 1993). In these rheumatoid models, non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to reduce development of arthritis (Bendele *et al*., 1992; Takeshita *et al*., 1997). NSAIDs inhibit the formation of prostaglandins (PGs) which are important lipid mediators of the inflammatory process and are products of the cyclo-oxygenase (COX) pathway of arachidonic acid metabolism (Vane, 1971). Two isoforms of COX have been found, known as COX-1 and COX-2 (Hla & Neilson, 1992; Meade *et al*., 1993). COX-1 is expressed constitutively and high levels can be detected in most tissues and cells. In contrast, levels of COX-2 mRNA and protein are usually low or undetectable under basal conditions but are rapidly elevated during inflammation.

In recent years, there are evidences that in some conditions, COX-1 produces PGs that contribute to inflammation. For example, significant anti-inflammatory effects are only observed at doses of the drugs that inhibit COX-1 in carrageenan-induced inflammation (Wallace *et al*., 1998) and in granulomatous tissue air pouch model (Gilroy *et al*., 1998). It should also be added that COX-1 deficient mice have reduced platelet aggregation and a decreased inflammatory response to arachidonic acid, but not to tetradecanoyl phorbol acetate (Langenbach *et al*., 1995). However, the contribution of COX-1 to inflammation has not been completely established.

While screening for novel inhibitors of platelet aggregation without adverse gastrointestinal effects, we found FR122047, whose chemical structure is 1-[(4,5-bis(4-methoxyphenyl)-2-thiazoyl)carbonyl]-4-methylpiperazine hydrochloride (Dohi *et al*., 1993). *In vitro* studies, FR122047 is a selective and potent inhibitor of COX-1 (Ochi *et al*., 2000). To clarify the role for COX-1 in animal models of chronic inflammation, the anti-inflammatory effect of FR122047 has been characterized.

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Methods

Animals

Experiments were conducted in accordance with the ethical guidelines of International Association for the Study of Pain (Zimmermann, 1983). 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, Japan) were used at the age of 8 weeks. The animals were maintained in a group of five animals for at least 5 days on a 12-h light–dark cycle (light on from 0700 to 1900 h) in a controlled temperature ($23 \pm 1^{\circ}C$) and humidity ($55 \pm 5\%$) environment. The animals were given standard laboratory food and tap water *ad libitum* before the experiment.

Ex vivo *whole blood assay*

The technique of Brideau *et al*. (1996) was modified. Single oral doses of 0.032–3.2 mg kg^{-1} of drugs were administered to rats. Blood samples were taken at 1.5 h after dosing.

In COX-1 assay, blood was collected in siliconized tubes containing no anticoagulants. Aliquots of 500 μl were immediately transferred to siliconized microcentrifuge tubes. The tubes were vortexed and incubated at 37°C for 1 h to allow the blood to clot. Reactions were terminated by the addition of 5 μl of indomethacin 1 mM, and serum was obtained by centrifugation at $12{,}000 \times g$ for 5 min at 4°C. A 100 μl aliquot of serum was mixed with 400 μl methanol for protein precipitation. The supernatant was obtained and was assayed for thromboxane (TX) B_2 by radioimmunoassay (Amersham, Buckinghamshire, U.K.).

In COX-2 assay, blood was collected in heparinized tubes. Aliquots of 500 μl blood were immediately transferred to siliconized microcentrifuge tubes, and were incubated for 15 min at 37°C. This was followed by incubation of the blood with 10 μl lipopolysaccharide (LPS) (Sigma, St. Louis, MO, U.S.A., #L-2630 from *E. coli* serotype 0111:B4, 100 μg ml^{-1} final concentration, in phosphate-buffered saline) for 24 h at 37°C for induction of COX-2. Reactions were terminated by the addition of 5 μl of indomethacin 1 mM, and the blood was centrifuged at $12{,}000 \times g$ for 5 min at 4°C to obtain plasma. A 100 μl aliquot of plasma was mixed with 400 μl methanol for protein precipitation. The supernatant was obtained and assayed for PGE_2 by radioimmunoassay (Amersham, Buckinghamshire, U.K.).

Induction of type II collagen-induced arthritis

Type II collagen (CII) isolated and purified from bovine articular cartilage was purchased from Collagen Research Center (Tokyo, Japan) and dissolved overnight at 4°C in 0.01 M acetic acid at a concentration of 2 mg ml^{-1}. The solution was emulsified in an equal volume of incomplete Freund's adjuvant (ICFA, Difco Laboratories, Detroit, MI, U.S.A.). Each rat was immunized with 0.5 ml of the cold emulsion (0.5 mg CII) by several intradermal injections on the back and one or two injections into the base of the tail (Inamura *et al*., 1988). They were challenged with 0.2 ml of the emulsion (0.2 mg CII) into the base of the tail on day 7 after immunization. The drugs were given orally once a day prophylactically from day 1 to day 24 after the first CII immunization. For the time course study, paw volume was measured before and 7, 10, 14, 18, 21 and 24 days after the first immunization with the Volume Meter TK-105 (Neuroscience, Tokyo, Japan), and oedema was expressed as the increase in paw volume (Δ ml) after CII immunization relative to the pre-immunization value for each animal. The anti-inflammatory effect was expressed as the difference in paw oedema compared with that of vehicle-treated CIA-control rats.

Induction of adjuvant-induced arthritis

A suspension of heat-killed and dried Mycobacterium tuberculosis H37 RA (0.5 mg; DIFCO, Detroit, MI, U.S.A.) in 0.05 ml liquid paraffin was administered by intradermal injection into the plantar surface of the right hind paw at day 0 to induce adjuvant arthritis (Newbould, 1963; Walz *et al*., 1971). The drugs were given orally once a day prophylactically from day 1 to day 24. Paw volume was measured before and 24 days after adjuvant injection with the Volume Meter TK-105, and the anti-inflammatory effect was expressed as the difference in paw oedema compared with that of vehicle-treated AIA-control rats.

Biochemical measurements in inflamed rat paw

The technique of Opas *et al*. (1987) was used. At day 24 after immunization, rats were euthanized by CO_2 inhalation and both hind paws were amputated. The paws were then placed immediately into n-hexane cooled by dry ice–acetone for 30 s. Frozen paws were then stored at −70°C until needed for extraction of arachidonic acid metabolites.

Frozen paw tissue was homogenized under cooling in 5 ml extraction buffer (75% methanol, 25% 0.1 M sodium acetate, adjusted to pH 3 with HCl). The extracted tissue was centrifuged at $1500 \times g$ for 10 min at 4°C. The resulting supernatant fluid was filtered through gauze and diluted with distilled water to a final concentration of 15% methanol. This solution was applied to a C_{18} Sep-Pak cartridge (Waters, Milford, MA, U.S.A.) that was prewashed with 10 ml of methanol, distilled water and 15% methanol. After loading Sep-Pak, the columns were sequentially washed with 5 ml of 15% methanol, distilled water and petroleum ether. The samples were eluted with 2 ml of methyl formate (Powell, 1980; 1982), evaporated under nitrogen gas, dissolved in 1 ml phosphate-buffered saline and assayed for PGE_2 and TXB_2 by radioimmunoassay (Amersham, Buckinghamshire, U.K.).

The efficiencies of recovery as determined by injection of radiolabeled PGE_2 and TXB_2 into amputated paws were as follows (mean per cent ± s.e.mean, $n = 3$): PGE_2, $42.0 \pm 2.1\%$; and TXB_2, $46.5 \pm 2.5\%$.

Gastric damage in chronic inflammation

The ulcerogenic activity was expressed in CIA rats with prophylactic treatment of drugs on day 24. After the rats were euthanized by CO_2, the stomachs were removed and placed in 2% formalin (Kanto Chemical, Tokyo, Japan). The stomach was opened by cutting along the greater curvature, and the lesion 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).

Drugs

Drugs used were indomethacin (Sigma, St. Louis, MO, U.S.A.) and FR122047 (Fujisawa, Osaka, Japan). These drugs were suspended and diluted in 0.5% methylcellulose.

Statistical analysis

The results were expressed as mean ± s.e.mean. Statistical significance was analysed using the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The difference between groups was considered statically significant when $P<0.05$. ED_{50} values and 95% confidence limits (95% C.L.) were calculated from the dose–per cent inhibition relations by computer log–linear analysis (Litchfield & Wilcoxon, 1949).

Results

Ex vivo *whole blood assay*

Inhibition curves for FR122047 and indomethacin on serum TXB_2 levels (COX-1) and LPS-induced PGE_2 production (COX-2) in the rats are shown in Figure 1. FR122047 inhibited TXB_2 production in coagulated blood (COX-1) with ED_{50} value (95% C.L.) of 0.059 (0.001–0.30) mg kg^{-1} in a dose-dependent manner. In contrast, FR122047 at maximum dose of 3.2 mg kg^{-1} showed only 34.5% inhibition for COX-2. FR122047 is more selective for COX-1. Indomethacin inhibited both COX-1 and COX-2 with approximately equal potency with ED_{50} values (95% C.L.) of 0.57 (0.16–2.2) and 0.33 (0.003–27) mg kg^{-1}, respectively.

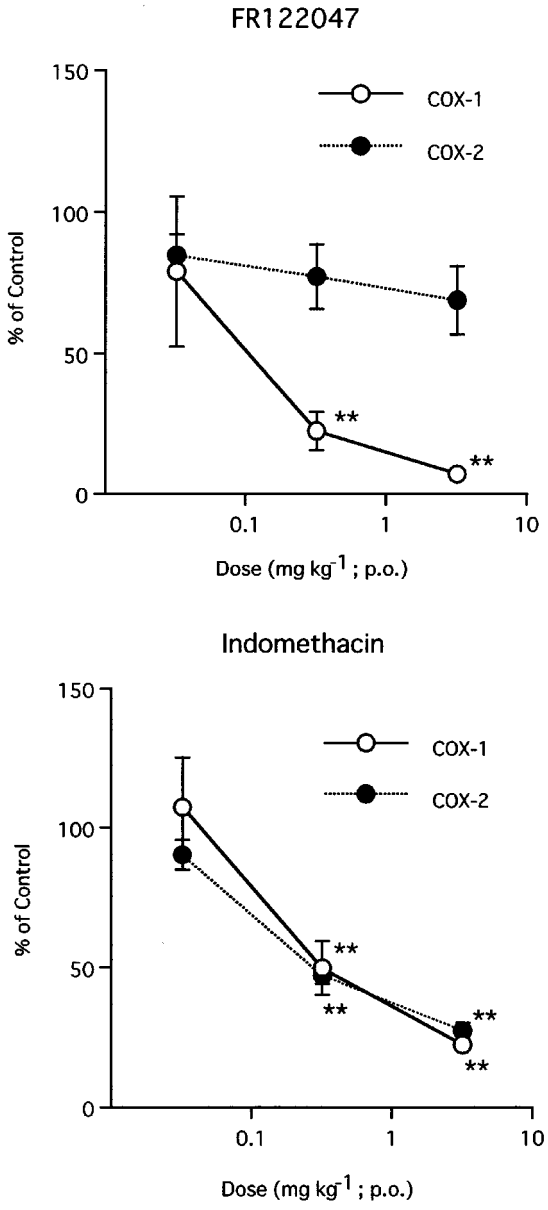

Figure 1 Effects of FR122047 on the activity of COX-1 and COX-2 in rat whole blood assay *ex vivo*. TXB_2 production in coagulated blood (COX-1) and PGE_2 production in LPS-treated blood (COX-2) at 1.5 h after oral dosing are shown. Results are given as a percentage of control COX activity. Significantly different from the control, $^{**}P<0.01$. Values are mean ± s.e.mean, $n=5$.

Anti-inflammatory effect of FR122047 in CIA rat model

Figure 2 shows the anti-inflammatory effect of FR122047 in CIA rats. The oedema in CIA rat paws on day 24 was 0.79 ± 0.03 ml. Oral administration of FR122047, at doses ranging from 0.032 to 3.2 mg kg^{-1}, to CIA rat resulted in significant inhibition of the paw oedema. After 24 days of prophylactic treatment, paw oedema was reduced in the FR122047 and indomethacin treated animals with ED_{50} values (95% C.L.) of 0.56 (0.098–9.4) and 0.16 (0.021–0.73) mg kg^{-1} in a dose-dependent manner, respectively (Figure 3).

Effect of FR122047 on the formation of prostanoids in CIA rat paws

Oral administration of FR122047 (0.032–3.2 mg kg^{-1}) dose dependently reduced the formation of PGE_2 and TXB_2 in CIA rat paws with ED_{50} values (95% C.L.) of 0.24 (0.015–2.0) and 0.13 (0.022–0.45) mg kg^{-1}, respectively (Figure 4). Indomethacin also showed a dose-dependent inhibition of the formation of PGE_2 and TXB_2 in CIA rat paws with ED_{50} values (95% C.L.) of 0.086 (0.001–0.43) and 0.077 (0.020–0.20) mg kg^{-1}, respectively.

Effect of FR122047 in AIA rat model

Prophylactic treatment in AIA rats with FR122047 at doses up to 3.2 mg kg^{-1} (p.o.) did not show an anti-inflammatory effect for adjuvant-injected paws and adjuvant-uninjected paws (Figure 5). On the other hand, oral administration of indomethacin (0.032–3.2 mg kg^{-1}) dose dependently inhibited both paw oedema in AIA rats. Oral administration of

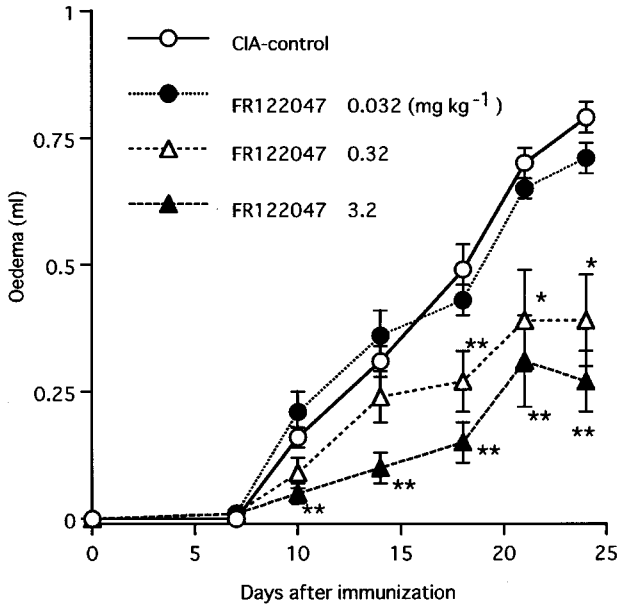

Figure 2 Prophylactic effect of FR122047 on type II collagen-induced arthritic rat paw oedema. FR122047 at doses 0.032, 0.32 and 3.2 mg kg^{-1} and vehicle-treated CIA-control were given orally once a day prophylactically from day 1 to day 24 after the first CII immunization (0.5 mg). Significantly different from the CIA-control, $*P<0.05$, $**P<0.01$. Values are mean ± s.e.mean, $n=5$.

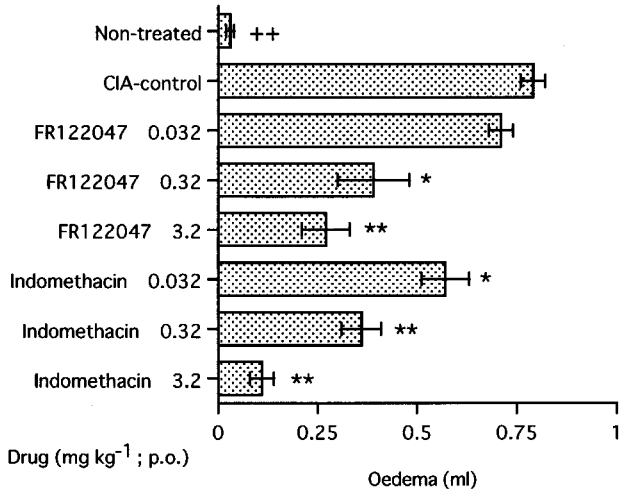

Figure 3 Anti-inflammatory effect of drugs on type II collagen-induced arthritic rat paw oedema. Drugs were given orally once a day prophylactically from day 1 to day 24 after the first CII immunization (0.5 mg). Significantly different from the CIA-control, $++P<0.01$, $*P<0.05$, $**P<0.01$. Values are mean ± s.e.mean, $n=5$.

indomethacin, but not FR122047, dose dependently reduced the levels of PGE_2 and TXB_2 in AIA rat paws (Table 1).

Gastric damage of drugs in CIA rats

The gastric damage of FR122047 was evaluated after its chronic administration to rats in CIA (Table 2). Drugs were administered orally once a day from day 1 to day 24 after the first CII immunization. Prophylactic treatment in CIA with

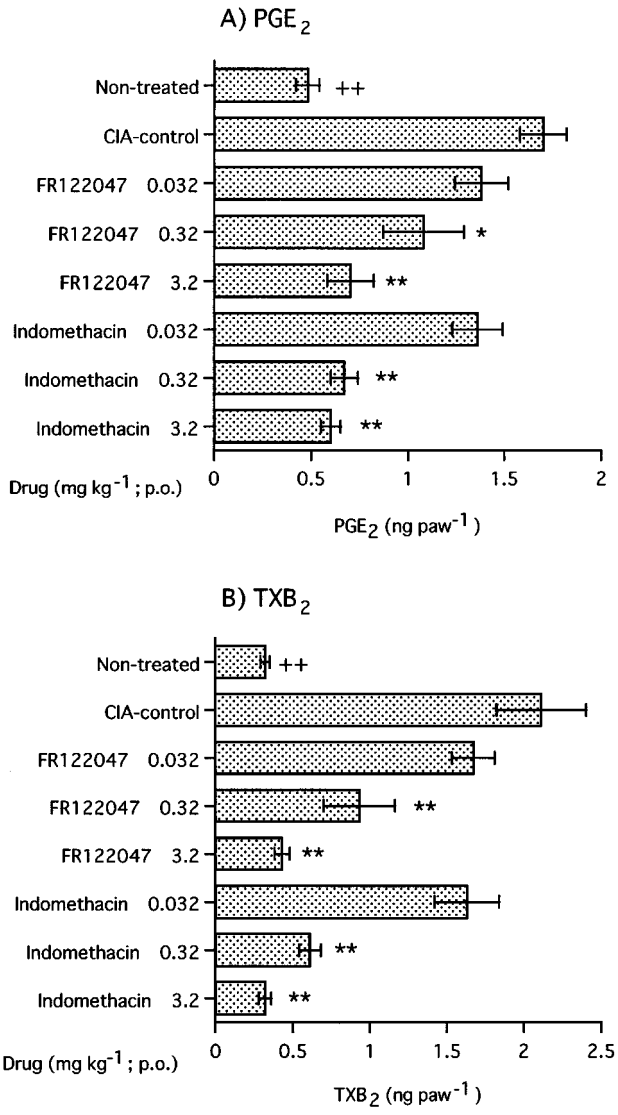

Figure 4 Effects of FR122047 on the formation of PGE_2 (A) and TXB_2 (B) in type II collagen-induced arthritic rat paws. Drugs were given orally once a day prophylactically from day 1 to day 24 after the first CII immunization (0.5 mg). Rats were euthanized by CO_2 inhalation 24 days after the first CII immunization, and PGE_2 and TXB_2 in the CIA rat hind paws were extracted and analysed by radioimmunoassay. Significantly different from the CIA-control, $++P<0.01$, $*P<0.05$, $**P<0.01$. Values were corrected for recovery efficiency and expressed as ng paw^{-1} ± s.e.mean, $n=5$.

FR122047 at doses between 0.032–3.2 mg kg^{-1} did not induce any mucosal lesions. In contrast, indomethacin at a dose of 3.2 mg kg^{-1} induced marked gastric lesions in two of five rats.

Discussion

The purpose of this paper is to evaluate the contribution of isoforms of COX to chronic inflammation in rat CIA and AIA. Few attempts have so far been made at defining a role for COX-1 in inflammation (Smith *et al.*, 1998; Wallace *et al.*, 1999). We previously reported that FR122047 is greater than

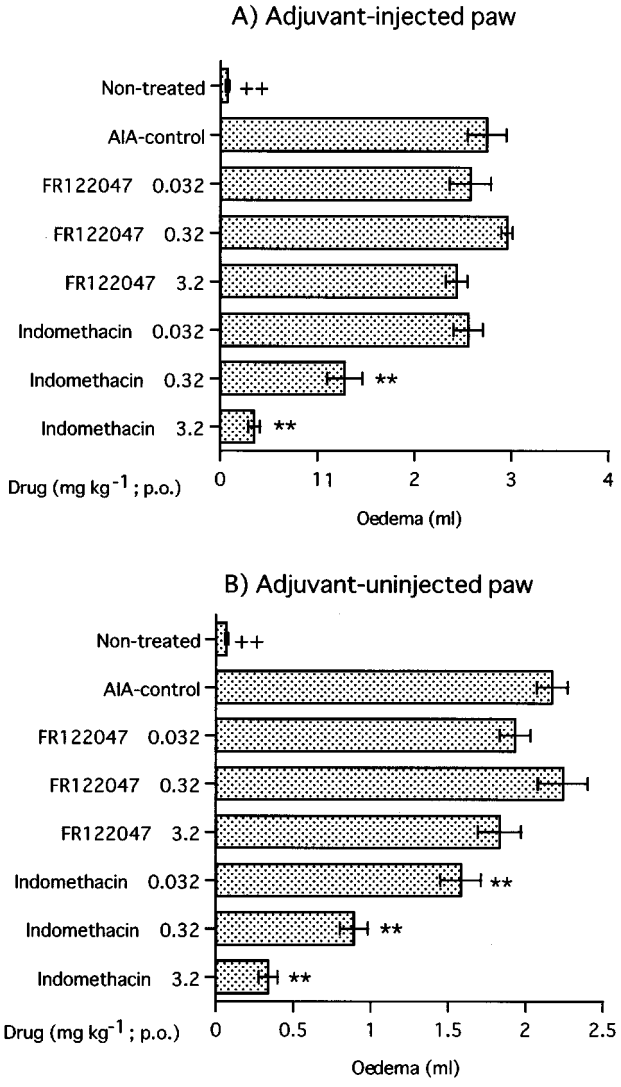

Figure 5 Anti-inflammatory effect of drugs on adjuvant-induced arthritic rat paw oedema. Drugs were given orally once a day prophylactically from day 1 to day 24 after adjuvant injection. Significantly different from the AIA-control, $++P<0.01$, $**P<0.01$. Values are mean ± s.e.mean, $n=5$.

2300 times more potent as an inhibitor of COX-1 compared with COX-2 in the recombinant human enzyme assay (Ochi *et al*., 2000). In the present study, we found that FR122047 at single doses of 0.032–3.2 mg kg^{-1} selectively inhibits *ex vivo* rat whole blood COX-1, indicating that it is orally active, in good agreement with its *in vitro* effects on human COX-1. To discuss the role for COX-1 in rat CIA model, we evaluated the anti-inflammatory effect of selective COX-1 inhibitor FR122047 in this arthritic model. Oral administration of FR122047, at doses ranging from 0.032 to 3.2 mg kg^{-1}, showed an anti-inflammatory effect in Lewis rats with CIA, one of the animal models for human rheumatoid arthritis. To demonstrate the relationship between the oedema and the production of PGs in the inflamed rat paws induced by CII, we extracted PGs, PGE_2 and TXB_2, from CIA rat paws after prophylactic treatment with FR122047. Oral administration of FR122047, a highly selective COX-1 inhibitor, dose dependently inhibited the formation of PGE_2 and TXB_2 in inflamed rat paws. The ED_{50} values of FR122047 for the inhibitory effects on PGE_2 and TXB_2 production in inflamed paws were almost the same as the ED_{50} value of FR122047 for its anti-inflammatory effect in CIA rat model. The results suggest modulation of the rat CIA model in the paw oedema through COX-1 inhibition. It is clear that COX-1 contributes to inflammation in this model.

While the inhibitory effect of FR122047 on rat whole blood COX-1 with ED_{50} value of 0.059 mg kg^{-1} was 10 times more potent than that of indomethacin with ED_{50} value of 0.57 mg kg^{-1}, the anti-inflammatory effect of FR122047 in rat CIA model was less potent than that of indomethacin, which inhibits both COX-1 and COX-2 activity to a similar degree. This discrepancy on the comparative effects of FR122047 and indomethacin suggests that an other subtype of COX has a role in the inflammation induced by CII as well as COX-1. We have previously reported that FR140423 (3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)-phenyl]pyrazole), which selectively inhibits COX-2 compared with COX-1 *in vitro* studies (Ochi *et al*., 1999), shows an anti-inflammatory effect in rat CIA and suppresses the levels of PGE_2 and TXB_2 in CIA rat paws (Ochi & Goto, 2001). These evidences lead to the conclusion that PGE_2 and TXB_2 formed by both COX isoforms (1 and 2) play important roles in rat CIA model.

On the other hand, FR122047 at doses up to 3.2 mg kg^{-1} had no effect on oedema and the formation of PGE_2 and

Table 1 Effects of drugs on the formation of PGE_2 and TXB_2 in adjuvant-induced arthritic rat paws

Drug (mg kg^{-1}; p.o.)		*Injected paw* (ng paw^{-1})		*Uninjected paw* (ng paw^{-1})	
		PGE_2	*TXB_2*	*PGE_2*	*TXB_2*
Non-treated		0.9±0.6++	0.07±0.01++	3.2±0.4++	0.11±0.01++
AIA-control		88.3±26.4	8.90±1.94	22.1±2.1	0.39±0.05
FR122047	0.032	82.4±12.5	7.79±1.59	21.6±3.1	0.34±0.08
	0.32	84.7±11.2	6.10±1.14	23.0±1.9	0.32±0.04
	3.2	65.6±4.7	7.25±1.68	19.7±2.2	0.36±0.04
Indomethacin	0.032	68.8±11.7	5.53±1.41	17.3±2.3	0.34±0.04
	0.32	58.9±7.9	3.83±0.60*	8.6±0.6**	0.25±0.03*
	3.2	19.0±2.5*	1.94±0.48**	5.4±0.5**	0.13±0.03**

Drugs were given orally once a day prophylactically from day 1 to day 24 after adjuvant injection. Rats were euthanized by CO_2 inhalation 24 days after adjuvant injection, and PGE_2 and TXB_2 in the AIA rat hind paws were extracted and analysed by radioimmunoassay. Significantly different from the AIA-control, $++P<0.01$, $*P<0.05$, $**P<0.01$. Values were corrected for recovery efficiency and expressed as ng paw^{-1} ± s.e.mean, $n=5$.

Table 2 Gastric damage of the drugs in type II collagen-induced arthritic rats

Drug (mg kg^{-1}; p.o.)		*Lesion index*	*Incidence* (%)
Non-treated		0.0±0.0	0
CIA-control		0.0±0.0	0
FR122047	0.032	0.0±0.0	0
	0.32	0.0±0.0	0
	3.2	0.0±0.0	0
Indomethacin	0.032	0.0±0.0	0
	0.32	0.0±0.0	0
	3.2	1.0±0.6	40

Drugs were administered orally once a day prophylactically from day 1 to day 24 in CII-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 mean ± s.e.mean, $n = 5$.

TXB_2 in rat AIA model. Indeed, FR122047 at doses of 0.032 – 3.2 mg kg^{-1} showed the selective inhibition of rat COX-1 but not COX-2. Therefore, the lack of effect of FR122047 in rat AIA model confirms that COX-1 is not responsible for PGs production in this arthritic model. In contrast, indomethacin, which inhibits both COX-1 and COX-2, showed an anti-inflammatory effect following the inhibition of PGE_2 and TXB_2 production in inflamed paws. Anderson *et al*. (1996) reported that COX-2 mRNA and protein are elevated in AIA rat paws without significant change in COX-1 expression. The development of AIA is associated with the upregulation of PGE_2 produced exclusively by COX-2 (Sano *et al*., 1992). In addition to these, well-known selective COX-2 inhibitors, NS-398 (*N*-[2-cyclohexyloxy-4-nitrophenyl]methanesulphonamide), celecoxib (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzensulfonamide) and rofecoxib (4-(4′-methylsulphonylphenyl)-3-phenyl-2-(5*H*)-furanone), show anti-inflammatory effects in rat AIA in a dose-dependent manner (Futaki *et al*., 1993; 1994; Penning *et al*., 1997; Chan *et al*., 1999). Additionally, a similar result is obtained with other selective COX-2 inhibitor FR140423 (Ochi & Goto, 2000). These results suggest that COX-2, but not COX-1, plays a role in rat AIA model. COX-1 and COX-2 differ in cellular source and distribution of intracellular activity. Prostanoids synthesis through COX-1 and COX-2 involves different arachidonate substrate polls coupled to different extracellular stimuli and different phospholipase systems (Reddy & Herschman, 1996). Furthermore, platelet TX is synthesized by COX-1 (Funk *et al*., 1991; Reiter *et al*., 2001), but an alternative source of TX formation by alveolar macrophages is COX-2 (Lee *et al*., 1992). FR122047 potently inhibits platelet aggregation *ex vivo* induced by arachidonic acid and collagen with ED_{50} values of 0.28 and 0.53 mg kg^{-1}, respectively (Dohi *et al*., 1993). The cause of this discrepancy on the anti-inflammatory effects of FR122047 in rat chronic models of arthritis is probably because the roles of platelet in these animal models of arthritis are different in these experimental conditions. To clarify the difference between CIA and AIA requires further investigation.

The unwanted side-effects of NSAIDs, which cause the most damage to the stomach, are due to their ability to inhibit COX-1 (Vane, 1994). However, COX-1 deficient mice spontaneously have no gastric pathology, even though their gastric PGE_2 levels are about 1% of the levels observed in wild-type mice (Langenbach *et al*., 1995). Wallace *et al*. (2000) concluded that inhibition of both COX-1 and COX-2 is required for NSAID-induced gastrointestinal toxicity. In healthy rats, the selective COX-1 inhibitor SC-560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethylpyrazole)does not cause mucosal injury, although formation of gastric 6-keto $PGF_{1\alpha}$ and platelet TXB_2 is substantially inhibited (Gretzer *et al*., 2001). In this study, the present authors experienced that selective COX-1 inhibitor FR122047 given in daily oral doses of 0.032 to 3.2 mg kg^{-1} for 24 days to CIA rats results in no visible gastric mucosal lesions unlike non-selective COX inhibitor indomethacin. COX-1 inhibition resulted in reduced gastric blood flow, whereas COX-2 inhibition leads to increased leukocyte adherence to the vascular endothelium (Wallace *et al*., 2000). Further studies are needed to discover whether treatment of FR122047 results in a reduction in gastric blood flow.

In conclusion, the treatment of CIA with FR122047, a selective inhibitor of COX-1, produces an anti-inflammatory effect following the inhibition of PGE_2 and TXB_2 production at a site of inflammation. This compound would be a useful tool for studying the physiological role of COX-1.

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