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The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models

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Abstract

The pharmacological profile of the analgesic agent, 1-[(4,5-bis(4-methoxyphenyl)-2-thiazoyl)carbonyl]-4-methylpiperazine hydrochloride (FR122047), was investigated. In recombinant human cyclooxygenase enzyme assays, the inhibition of prostaglandin E_2 formation by FR122047 was 2300 times more selective for cyclooxygenase-1 than cyclooxygenase-2. Oral administration of FR122047 (3.2–100 mg/kg) dose dependently reduced the phase 2 response (10–60 min) of the formalin test in rats. This effect was 3 times less potent than that of indomethacin. FR122047 (1–32 mg/kg; p.o.) showed a dose-dependent analgesic effect against the acetic acid-induced writhing response in mice. Furthermore, FR122047 (0.01–10 mg/kg, p.o.) inhibited the increase in 6-keto prostaglandin $F_{1\alpha}$ level in acetic acid-injected mouse peritoneal cavity. However, a selective cyclooxygenase-2 inhibitor, NS-398, had no effect in these cyclooxygenase-1 sensitive pain models. These results suggest that FR122047, a selective cyclooxygenase-1 inhibitor, shows an analgesic effect in chemical nociceptive models and may be a useful analgesic agent.

Keywords: FR122047; Cyclooxygenase-1; Formalin test; Acetic acid-induced writhing

1. Introduction

Prostaglandins have been shown to be important mediators of pain (Collier and Schneider, 1972). Because nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and ibuprofen, which inhibit the production of prostaglandins, show analgesic effects, prostaglandins have long been associated with pain (Doherty, 1987).

Cyclooxygenase is the rate-limiting enzyme that catalyzes the conversion of arachidonic acid to prostaglandins. There are two isoforms of cyclooxygenase, constitutive cyclooxygenase-1 and inducible cyclooxygenase-2 (Hla and Neilson, 1992; Meade et al., 1993). The two isoforms are regulated and expressed differently in various cells and tissues. In general, cyclooxygenase-1 is detectable in most normal tissues, including human stomach, kidney and platelets. Cyclooxygenase-2 is not detected in normal tissues; however, it is rapidly induced in response to inflammatory stimuli by endotoxin, mitogens and cytokines (Hla and Neilson, 1992; Xie et al., 1992; Cao et al., 1996). Cyclooxygenase-2 selective inhibitors such as NS-398 and JTE-522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide) have now been introduced (Futaki et al., 1994; Wakitani et al., 1998). Selective inhibitors of cyclooxygenase-2 were reported to show analgesic effects on yeast- and carrageenan-induced hyperalgesic models (Matsushita et al., 1997; Yamamoto and Nozaki-Taguchi, 1997; Dirig et al., 1998). On the other hand, the analgesic effects of selective cyclooxygenase-2 inhibitors in the formalin test and in the writhing model were weak (Dirig et al., 1997; Matsushita et al., 1997). Prostaglandins have been shown to be important mediators of pain; however, the responsible cyclooxygenase isoform has not yet been identified in such models.

While screening for novel inhibitors of platelet aggregation, we found FR122047, whose chemical structure is 1-[(4,5-bis(4-methoxyphenyl)-2-thiazoyl)carbonyl]-4-methylpiperazine hydrochloride (Dohi et al., 1993). FR122047 is a structurally unique cyclooxygenase inhibitor that is concentrated in platelets.

We now report that FR122047 is a selective and potent inhibitor of cyclooxygenase-1, and that the effects of FR122047 in the rat formalin test, in which the intradermal

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injection of formalin induces an acute nociceptive response, and on acetic acid-induced writhing in mice are comparable to those of indomethacin and NS-398. The role of the two isoforms in the induction of chemical nociception is also discussed.

2. Materials and methods

2.1. Animals

Ethical guidelines for the experimental use of animals were followed (Zimmermann, 1983). In addition, the experimental work was approved by the Fujisawa Pharmaceutical Animal Experiment Committee for Animal Experimentation.

Male Sprague–Dawley rats (160–200 g, Japan SLC, Hamamatsu, Japan) at age of 6 weeks and male ddY mice (25–35 g, Japan SLC) at the age of 6 weeks were used. The animals were maintained on a 12-h light–dark cycle (light on from 0700 to 1900 h) with controlled temperature (23 ± 1°C) and humidity (55 ± 5%) and standard laboratory food and tap water ad libitum before the experiments.

2.2. Drugs

Indomethacin was obtained from Sigma (St. Louis, MO, USA). NS-398 (*N*-[2-cyclohexyloxy-4-nitrophenyl]-methanesulfonamide) was obtained from Cayman (Ann Arbor, MI, USA). FR122047 was synthesized at Fujisawa Pharmaceutical (Osaka, Japan).

2.3. Human recombinant cyclooxygenase-1 and cyclooxygenase-2 enzyme assay

Human recombinant cyclooxygenase-1 and cyclooxygenase-2 were expressed in Chinese hamster ovary cells. The appropriate cyclooxygenase enzyme (1 μg for cyclooxygenase-1 and/or 3 μg for cyclooxygenase-2) was preincubated in 100 mM Tris–HCl buffer (pH 7.3) containing hematin (2 μM) and tryptophan (5 mM) with drugs (0.001–100 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 prostaglandin E_2 production was measured by radioimmunoassay (Amersham, Buckinghamshire, England).

2.4. Formalin test in rats

Male Sprague–Dawley rats (five animals per group) were fasted for 16 h before the experiments. FR122047 (3.2–100 mg/kg), indomethacin (1–10 mg/kg) and NS-398 (10,100 mg/kg) were suspended and diluted in 0.5% methylcellulose, and administered orally in a volume of 5 ml/kg, 1 h before formalin injection. For formalin injection, the animals were gently restrained, and 0.1 ml of 5% formalin was injected subcutaneously into the plantar surface of the right hind paw. Time zero of the study was defined as injection of formalin. Immediately after the injection, the animal was placed in an open Plexiglas box, which permitted observation. Reaction to pain was quantified by counting the number of flinching/shaking of the injected paw (Wheeler-Aceto et al., 1990). Animals were observed as previously described for 60 min.

2.5. Acetic acid-induced writhing in mice

Male ddY mice were fasted for 16 h before the experiments. FR122047 (1–32 mg/kg), indomethacin (0.32–32 mg/kg) and NS-398 (1–100 mg/kg) were suspended and diluted in 0.5% methylcellulose, and administered orally in a volume of 10 ml/kg, 1 h before acetic acid injection. The writhing reaction was induced by an intraperitoneal injection of 0.6% acetic acid (20 ml/kg) into the mice. Three minutes later, the number of writhings was counted for 10 min.

2.6. Prostaglandin production in mice peritoneal cavity

At 3 min after injection of acetic acid, the mice were killed by cervical dislocation. The abdominal skin was immediately peeled back and the mice were injected i.p. with 2.5 ml of phosphate-buffered saline (Nikken Bio Medical Laboratory, Kyoto, Japan) containing 10 μM indomethacin. After gentle massage, the peritoneal cavity was exposed through a 10–15 mm incision and the lavage fluid was pipetted off and transferred to a microcentrifuge tube. The samples were centrifuged at 3,000 rpm for 10 min at 4°C and the supernatant was then transferred to a 15-ml polypropylene tube containing 200 μl of 1 N HCl to facilitate extraction. Five milliliters of ethyl acetate was added to all tubes, which were then vortexed for 10 s and placed on ice. The organic (upper) layer (4 ml) was transferred to a clean 15-ml polypropylene tube, evaporated under nitrogen gas, dissolved in 0.4 ml phosphate-buffered saline and assayed for prostaglandin, 6-keto prostaglandin $F_{1\alpha}$, by radioimmunoassay (Amersham).

Recovery, as determined by the injection of 6-keto prostaglandin $F_{1\alpha}$ into the peritoneal cavity, was 18.0 ± 5.0% (mean percent ± S.E.M., $n = 4$).

2.7. Statistical analysis

The results are expressed as means ± S.E.M. Statistical significance was analyzed using the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. ED_{50} and IC_{50} values and 95% confidence limits (95% C.L.) were calculated from the dose-percent inhibition relations by computer log-linear regression analysis (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Effects of FR122047 on human recombinant cyclooxygenase-1 and cyclooxygenase-2 enzymes in vitro

Fig. 1 shows the effects of FR122047 on the activity of recombinant human cyclooxygenase-1 and cyclooxygenase-2. IC_{50} values for FR122047 were 0.028 ± 0.009 and 65 ± 19 μM for cyclooxygenase-1 and cyclooxygenase-2, respectively, which suggests that the inhibitory activity of FR122047 against cyclooxygenase-1 was 2300 times more potent than that against cyclooxygenase-2. The IC_{50} values for the reference compound, indomethacin, for cyclooxygenase-1 and cyclooxygenase-2 were 0.22 ± 0.03 and 0.58 ± 0.23 μM, respectively. NS-398 concentration dependently inhibited cyclooxygenase-2 with an IC_{50} value of 0.082 ± 0.014 μM, whereas NS-398 at concentrations up to 100 μM had no effect on cyclooxygenase-1 activity.

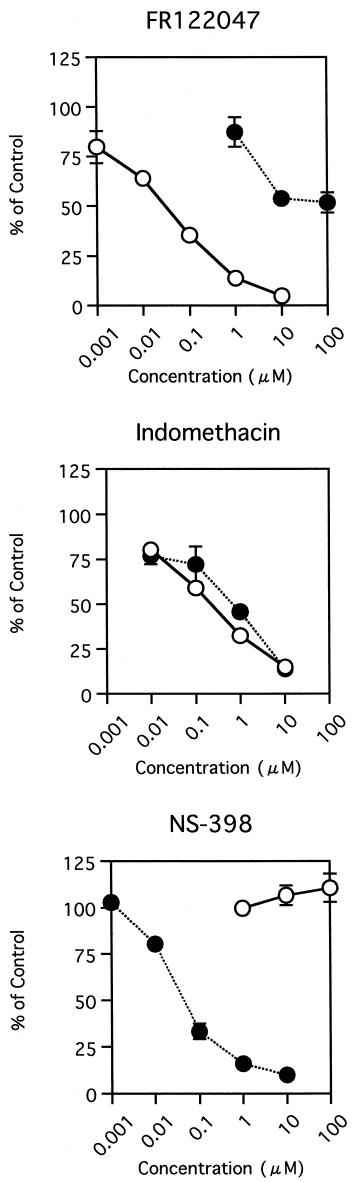

Fig. 1. Effects of FR122047 on the activity of cyclooxygenase-1 (open circle) and cyclooxygenase-2 (closed circle) in Chinese hamster ovary cells expressing the human recombinant enzymes. FR122047, indomethacin and NS-398 were incubated with cyclooxygenase for 5 min at 37°C before the addition of arachidonic acid (10 μM). Arachidonic acid was converted to prostaglandin E_2 after incubation of the reaction mixture for 5 min at 37°C. Results are given as percentages of control cyclooxygenase activity. Values are means ± S.E.M., $n = 3$.

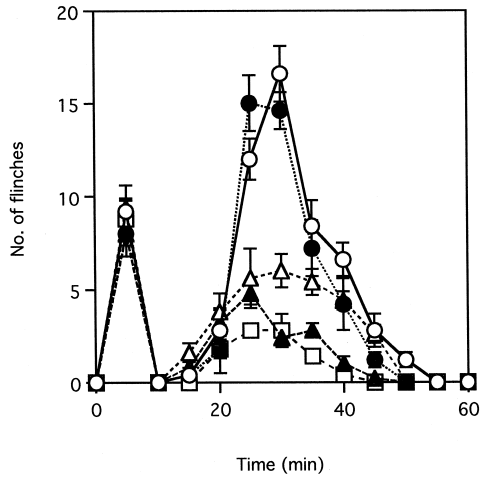

Fig. 2. Time course of antinociceptive effect of FR122047 in the formalin test in rats. Control (open circle) and FR122047 at 3.2 (closed circle), 10 (open triangle), 32 (closed triangle) and 100 (open square) mg/kg were administered p.o. 1 h before the formalin injection into the plantar surface of the right hind paw. The number of flinches per 5 min was plotted. Values are means ± S.E.M., $n = 3$.

3.2. Antinociceptive effect of FR122047 in the rat formalin test

The intraplantar injection of formalin resulted in a biphasic incidence of the flinching response (phase 1: 0–5 min, phase 2: 10–60 min) as shown in Fig. 2. The oral administration of FR122047 did not affect the phase 1 response, but dose dependently inhibited the duration of licking in the phase 2 response with an ED_{50} value (95% C.L.) of 17 (1.9–100) mg/kg. The antinociceptive effect of FR122047 in this animal model was 3 times less potent than that of indomethacin, which significantly suppressed the phase 2 response, but not the phase 1 response, with an ED_{50} value (95% C.L.) of 5.0 (0.68–23) mg/kg (Fig. 3). The formalin response after pretreatment with NS-398 (10,100 mg/kg, p.o.) did not differ from that of control animals. FR122047 alone, at the doses used in this assay, did not cause a behavioral change.

3.3. Analgesic effect of FR122047 on the acetic acid-induced writhing response in mice

FR122047 and indomethacin showed dose-dependent analgesic effects against the acetic acid-induced writhing

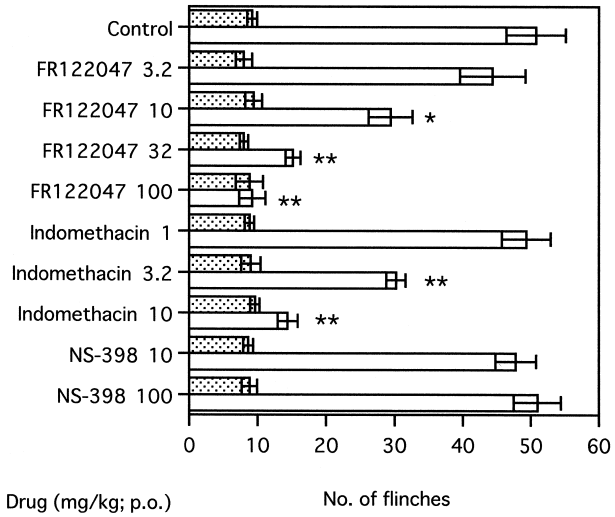

Fig. 3. Antinociceptive effect of FR122047 in the formalin test in rats. The number of flinches per phase 1 (dotted bar) and phase 2 (open bar) of the formalin test is shown. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$. Values are means ± S.E.M., $n = 5$.

behavior with ED_{50} values (95% C.L.) of 4.4 (0.15–49) and 3.3 (0.44–28) mg/kg, respectively (Fig. 4). However, the selective cyclooxygenase-2 inhibitor, NS-398, did not suppress the number of acetic acid-induced writhings. FR122047 alone, at the doses used in this assay, did not cause a behavioral change.

3.4. Effect of FR122047 on acetic acid-induced prostaglandin formation in mouse peritoneal cavity

The production of 6-keto prostglandin $F_{1\alpha}$ was measured in mouse peritoneal cavity 3 min after acetic acid injection. The level of 6-keto prostaglandin $F_{1\alpha}$ increased significantly 3 min after acetic acid injection (Fig. 5). FR122047 dose dependently inhibited the formation of 6-keto prostaglandin $F_{1\alpha}$. FR122047 10 mg/kg and indomethacin 10 mg/kg completely suppressed the increase of the 6-keto prostaglandin $F_{1\alpha}$ level in mouse peritoneal cavity. However, NS-398 at doses of 1–100 mg/kg showed only a weak inhibitory effect in this assay.

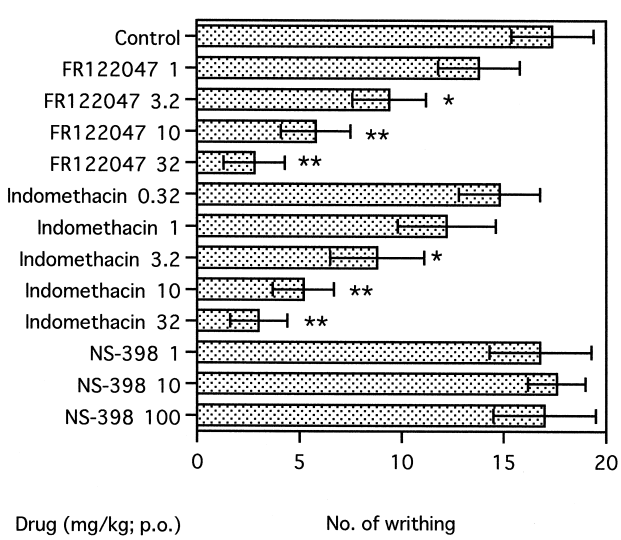

Fig. 4. Analgesic effect of FR122047 on acetic acid-induced writhing response in mice. Acetic acid (0.6%) was injected into peritoneal cavity in a volume of 20 ml/kg animal weight, 1 h after oral dosing of the drugs. Three minutes later, the total number of stretch responses (writhing) was counted for 10 min. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$. Values are means ± S.E.M., $n = 5$.

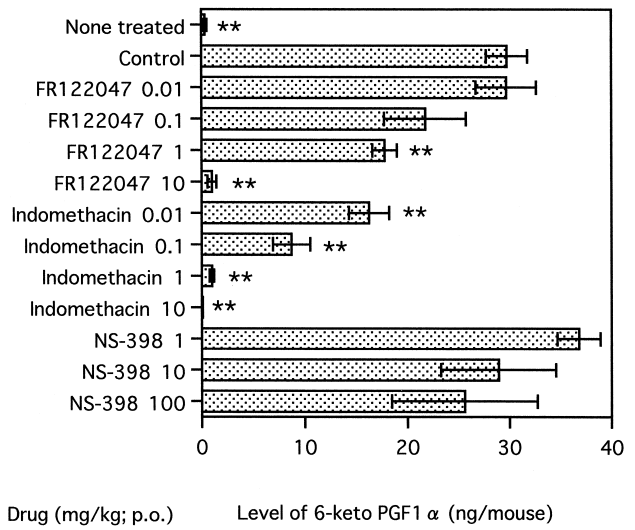

Fig. 5. Effect of FR122047 on acetic acid-induced 6-keto prostaglandin $F_{1\alpha}$ formation in mouse peritoneal cavity. The level of 6-keto prostaglandin $F_{1\alpha}$ was measured in the exudates obtained 3 min after the intraperitoneal injection of 0.6% acetic acid in a volume of 20 ml/kg animal weight. Drugs were administered orally 1 h before acetic acid injection. Significantly different from the control, $^{*}P < 0.05$, $^{**}P < 0.01$. Values were corrected for recovery efficiency and expressed as ng/mouse ± S.E.M., $n = 4$.

4. Discussion

In the recombinant human enzyme assay, FR122047 selectively inhibited cyclooxygenase-1 activity with an IC_{50} value of 0.028 ± 0.009 μM, while it showed a weak inhibitory effect on cyclooxygenase-2 activity. The inhibitory effect of FR122047 on cyclooxygenase-1 was 8 times more potent than that of indomethacin with an IC_{50} value of 0.22 ± 0.03 μM, while indomethacin inhibits both cyclooxygenase-1 and cyclooxygenase-2 with approximately equal potency. FR122047 potently inhibited the human platelet aggregation induced by arachidonic acid and collagen but not by ADP, and its action on platelets was a result of cyclooxygenase inhibition (Dohi et al., 1993). The production of thromboxane B_2, one of the metabolites of the arachidonic acid cascade via cyclooxygenase, following blood coagulation is used to induce cyclooxygenase-1 activity in the blood (Brideau et al., 1996; Matijevic-Aleksic et al., 1996). Judging from the

above, FR122047 is a selective and potent cyclooxygenase-1 inhibitor having anti-platelet actions.

The formalin test is a widely used model for studying pain (Wheeler-Aceto et al., 1990). The behavioral response to subcutaneous formalin was biphasic: phase 1 started immediately after formalin injection (0–5 min), followed by phase 2, which began after 10 min, with a maximum response typically observed at 30 min after the formalin injection (10–60 min). Hunskaar and Hole (1987) reported that the phase 1 response was due to a direct effect of formalin on nociceptors, whereas the phase 2 response involved inflammatory components with release of different pain-mediating substances. Oral administration of NSAIDs, which inhibit the production of prostaglandins, induced a dose-dependent inhibition of the phase 2 response, but not of the phase 1 response, in the formalin test (Shibata et al., 1989). In short, prostaglandins are involved in the phase 2 response evoked by subcutaneous injection of formalin. To clarify the role of the cyclooxygenase isoform in the nociceptive models, we used a selective cyclooxygenase-1 inhibitor, FR122047, a selective cyclooxygenase-2 inhibitor, NS-398, and a non-selective cyclooxygenase inhibitor, indomethacin, in chemical nociceptive models. In the formalin test, FR122047 and indomethacin had potent antinociceptive effects against phase 2, but not phase 1, of the flinching behavior. However, high doses of NS-398 did not inhibit either phase 1 or phase 2 nociceptive responses. Other selective cyclooxygenase-2 inhibitors, SC-58125 (1-[(4-methysulfonyl)phenyl]-3-tri-fluoromethyl-5-(4-fluorophenyl)pyrazole) and SC-236 (4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide), did not alter either phase 1 or phase 2 responses in the formalin test (Dirig et al., 1997). These data are consistent with our results. Thus, the present results suggest that cyclooxygenase-1, but not cyclooxygenase-2, is involved in the phase 2 response in the formalin test.

Next, we estimated the analgesic effect of FR122047 using the acetic acid-induced writhing model. The writhing model has been in general use for the evaluation of analgesic activity of NSAIDs (Schweizer et al., 1988; McCormack and Urquhart, 1995). FR122047 dose dependently inhibited the writhing response induced by acetic acid injection. The analgesic effect of FR122047 was as potent as that of indomethacin, whereas the selective cyclooxygenase-2 inhibitor, NS-398, did not show an analgesic effect. Thus, FR122047 may be an effective drug, which is similar to indomethacin for the treatment of acute pain, unlike cyclooxygenase-2 inhibitors. Intraperitoneal accumulation of prostaglandins, especially prostaglandin I_2, is reported to be intimately involved in the sensitization of pain receptors and the enhancement of the nociceptive writhing response (Juan, 1979), and the peak production of 6-keto prostaglandin $F_{1\alpha}$, a stable degradation product of prostaglandin I_2, is observed 3 min after acetic acid injection (Berkenkopf and Weichman, 1988). Prostaglandin I_2 could thus be the main nociceptive mediator for the acetic acid-induced writhing response in mice. Interestingly, prostaglandin I_2-deficient mice were recently shown to have a reduction in peripheral inflammation and nociception consistent with a role of prostaglandin I_2 in mediating nociception (Murata et al., 1997). In our preliminary experiments, prostaglandin levels were measured in mouse peritoneal cavity 3 min after acetic acid injection. The 6-keto prostaglandin $F_{1\alpha}$, prostaglandin E_2 and tromboxane B_2 levels were 29.7, 1.5 and 3.4 ng/mouse, respectively (data not shown). The level of 6-keto prostaglandin $F_{1\alpha}$ was higher than those of prostaglandin E_2 and tromboxane B_2. Thus, we measured 6-keto prostaglandin $F_{1\alpha}$ as a major prostaglandin in the peritoneal cavity during writhing. FR122047 at 10 mg/kg, a dose that inhibited the acetic acid-induced writhing response, completely suppressed the increase of 6-keto prostaglandin $F_{1\alpha}$ in acetic acid-injected mouse peritoneal cavity. However, NS-398 had no effect. These results indicate that 6-keto prostaglandin $F_{1\alpha}$, produced by constitutive cyclooxygenase-1, plays an important role in the induction of writhing. The inhibitory effects of FR122047 and indomethacin on the acetic acid-induced writhing response in mice had almost the same efficacy. However, the inhibitory effect of FR122047 on the formation of prostaglandin $F_{1\alpha}$ in acetic acid-injected mice peritoneal cavity was less potent than that of indomethacin. To clarify the reason for this discrepancy will require further investigation.

In conclusion, using selective inhibitors of cyclooxygenase-1 and cyclooxygenase-2 allowed us to make clear the different roles of cyclooxygenase-1 and cyclooxygenase-2 in pain. FR122047, which is a selective inhibitor of cyclooxygenase-1, has potent analgesic activity in cyclooxygenase-1-sensitive chemical nociceptive models such as the formalin test in rats and acetic acid-induced writhing in mice. The potent analgesic effects of FR122047 seem to be due to its inhibitory activity against cyclooxygenase-1.

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