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Anti-inflammatory and analgesic effects of a novel pyrazole derivative, FR140423

Takehiro Ochi *, Katsue Jobo-Magari, Atsuo Yonezawa, Kanae Matsumori, Takashi Fujii

Department of Immunology and Inflammation, Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical, 1-6, Kashima 2-chome, Yodogawa-ku, Osaka, 532-8514, Japan

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Abstract

The pharmacological profile of a novel and newly discovered non-steroidal anti-inflammatory and analgesic compound, 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole (FR140423), was investigated. In recombinant human cyclooxygenase enzyme assays, the inhibition of prostaglandin E_2 formation by FR140423 was 150 times more selective for cyclooxygenase-2 than cyclooxygenase-1. Oral administration of FR140423 dose dependently reduced carrageenin-induced paw edema and adjuvant arthritis. These effects were two- to three-fold more potent than those of indomethacin. Unlike indomethacin, FR140423 did not induce mucosal lesions in the stomach. FR140423 showed dose-dependent anti-hyperalgesic effects in the yeast-induced hyperalgesic model. This effect was five-fold more potent than that of indomethacin. Furthermore, FR140423 increased the pain threshold in non-inflamed paws and, unlike indomethacin, it produced an analgesic effect in the tail-flick test. These analgesic effects were blocked by the μ-opioid antagonist naloxone. These results suggest that FR140423, a selective cyclooxygenase-2 inhibitor, is a potent non-steroidal anti-inflammatory drug (NSAID) without gastrointestinal side effects and is a unique compound having morphine-like analgesic effects.

Keywords: FR140423; Anti-inflammation; Cyclooxygenase-2; Analgesia; Opioid; (Rat)

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and aspirin are widely used in the treatment of various diseases with inflammation, pain and fever, e.g., rheumatoid arthritis (Day, 1988). The common mechanism of action of NSAIDs is inhibition of prostaglandin formation as a result of inhibition of cyclooxygenase (Vane, 1971). However, the problem of side effects ascribed to cyclooxygenase inhibition after long-term administration of these drugs, such as gastrointestinal irritation and renal function abnormalities, has arisen in clinical trials (Fernandez et al., 1995; Lichtenberger et al., 1995). Therefore, the development of new NSAIDs without these side effects has long been awaited.

Recently, it has been reported that two isoforms of cyclooxygenase, constitutive cyclooxygenase-1 and inducible cyclooxygenase-2, exist (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 detected in most normal tissues, including human stomach, kidney and platelets, and is needed for normal physiological function such as cytoprotection and homeostasis. 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). These findings suggest that cyclooxygenase-2 is involved in the initiation and development of inflammatory disease, and that cyclooxygenase-1 is mainly concerned with the maintenance of normal essential physiological function in the gastrointestinal tract and kidney. The creation of selective cyclooxygenase-2 inhibitors, not the classically available non-selective cyclooxygenase inhibitors, is expected to lead to the development of ideal NSAIDs without side effects.

We have already reported on FR140423 (Fig. 1), a novel pyrazole derivative discovered by the screening of

* Corresponding author. Tel.: +81-6-6390-1297; Fax: +81-6-6304-5367

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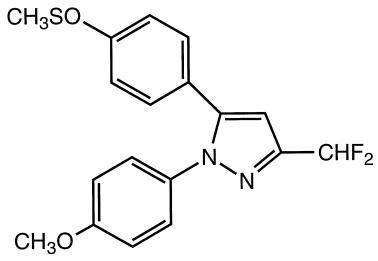

Fig. 1. Chemical structure of FR140423.

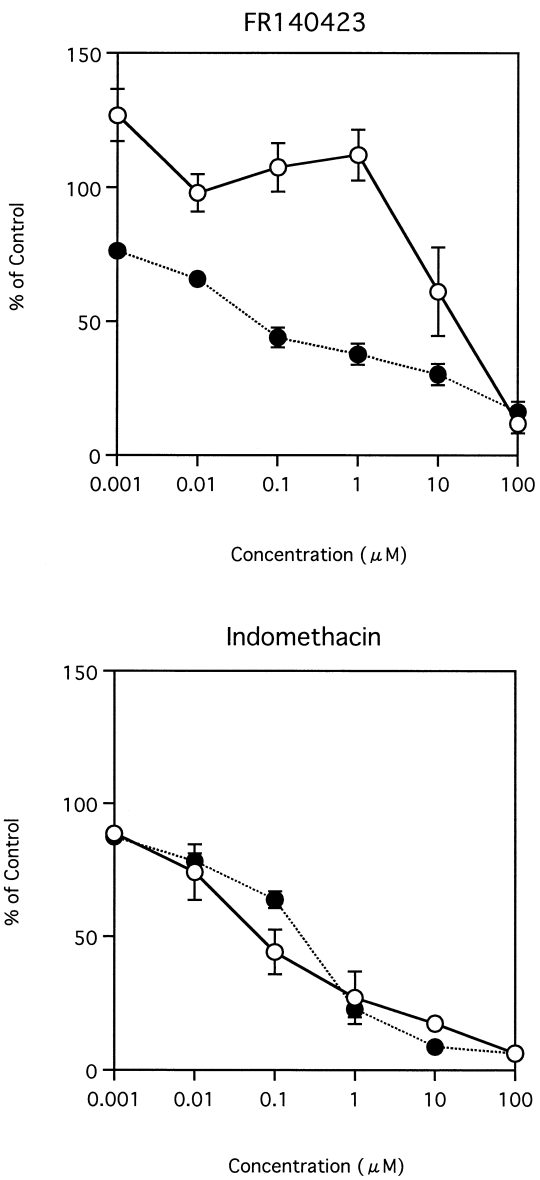

Fig. 2. Effects of FR140423 on the activity of cyclooxygenase-1 (open circle) and cyclooxygenase-2 (closed circle) in Chinese hamster ovary cells expressing the human recombinant enzymes. FR140423 and indomethacin were incubated with cyclooxygenase for 5 min at 37°C before addition of arachidonic acid (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 a percentage of control cyclooxygenase activity. $n = 3$. Values are means ± S.E.M.

compounds to find new anti-inflammatory drugs without side effects (Tsuji et al., 1997). In this paper, we discuss the relationship between its selective inhibition of cyclooxygenase-2 and its anti-inflammatory effects without ulcerogenic potential, and describe an analgesic effect that may also involve the activation of μ-opioid receptors.

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 reviewed by the Fujisawa Pharma-

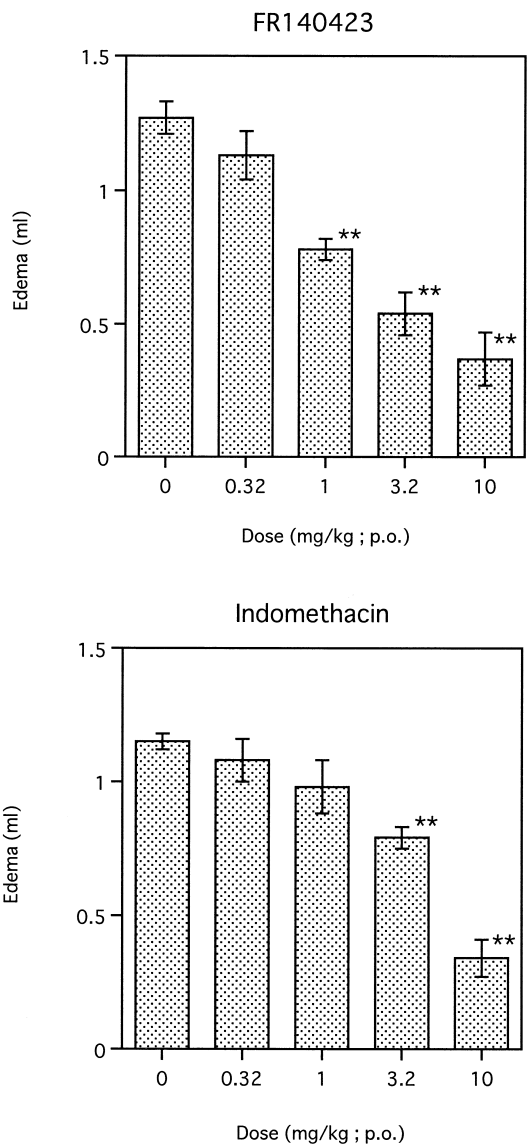

Fig. 3. Anti-inflammatory effect of FR140423 on carrageenin-induced paw edema in rats. Carrageenin (1 mg/paw) was injected into the planter surface of the right hind paw, 1 h after oral dosing of the drugs. Increases in paw volume, edema, were measured before and 3 h after carrageenin injection. Significantly different from the control, $** P < 0.01$. $n = 5$. Values are means ± S.E.M.

ceutical Animal Experiment Committee for Animal Experimentation.

Male Sprague–Dawley rats (160–200 g, Japan SLC, Hamamatsu, Japan) at the age of 6 weeks and female Lewis rats (140–180 g, Charles River Japan, Yokohama, Japan) at the age of 8 weeks were used. The animals were housed for at least 5 days in a controlled environment and were allowed food and water ad libitum.

2.2. Drugs

Indomethacin and naloxone HCl were obtained from Sigma (St. Louis, MO, USA). Morphine HCl was obtained from Dainippon Pharmaceutical (Osaka, Japan). FR140423 (3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole) was chemically 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

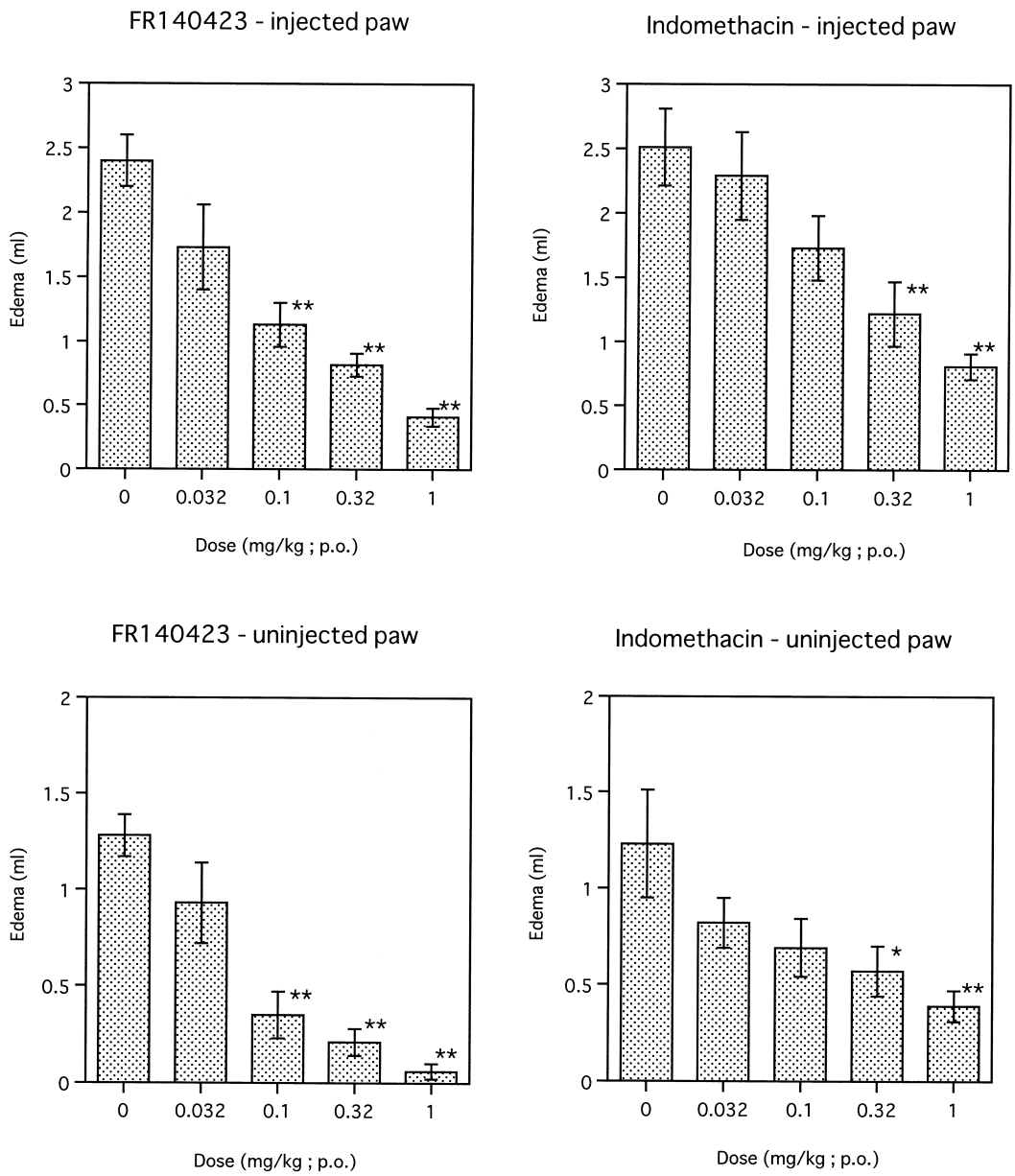

Fig. 4. Anti-inflammatory effect of FR140423 on adjuvant arthritic rat paws. Heat-killed and dried *Mycobacterium tuberculosis* H37 RA (0.5 mg/paw) was injected into the planter surface of the right hind paw. Drugs were given orally once a day prophylactically from day 1 to day 24. Increases in paw volume, edema, were measured before and on day 24 after adjuvant injection. Significantly different from the control, $* P < 0.05$, $** P < 0.01$. $n = 5$. Values are means ± S.E.M.

Table 1
Gastric ulcerogenicity of the drugs in rats

Drug (mg/kg; p.o.)		Ulcer index	Incidence (%)	UD_{50} (mg/kg; p.o.) (95% C.L.)
Control		0.0 ± 0.0	–	
FR140423	1	0.0 ± 0.0	0.0	
	3.2	0.0 ± 0.0	0.0	
	10	0.0 ± 0.0	0.0	> 100
	32	0.0 ± 0.0	0.0	
	100	0.0 ± 0.0	0.0	
Indomethacin	1	0.6 ± 0.6	20.0	
	3.2	1.2 ± 0.7	40.0	4.4
	10	1.8 ± 0.8	60.0	(0.93–13)
	32	3.8 ± 0.2	100.0	

Drugs were administered orally 5 h before the rats were euthanized. Visible gastric lesions were scored (score scales: petechiae = 1, erosion = 2, lesions between one and four = 3, lesions greater than five = 4). $n = 5$. Values are means ± S.E.M.

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. Carrageenin-induced paw edema

The carrageenin-induced paw edema model was used as previously described (Winter et al., 1962). Male Sprague–Dawley rats were fasted for 16 h before the experiment. Drugs (0.32–10 mg/kg) were suspended and diluted in 0.5% methylcellulose, and administered orally in a volume of 5 ml/kg, 1 h before carrageenin injection. Hind paw edema was induced in rats (five animals per group) by intradermal injection of 0.1 ml of 1% lambda-carrageenin (Sigma) in saline into the planter surface of the right hind paw. Paw volume was measured before and 3 h after carrageenin injection by using Volume Meter TK-105 (Neuroscience, Tokyo, Japan).

2.5. Adjuvant arthritis

Adjuvant arthritis was induced in female Lewis rats by intradermal injection into the plantar 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 (Newbould, 1963; Walz et al., 1971). The drugs (0.032–1 mg/kg), suspended and diluted in 0.5% methylcellulose, 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 volume compared with that of vehicle-treated control rats.

2.6. Gastric ulcergenic activity

Male Sprague–Dawley rats were fasted for 16 h prior to drug exposure. A total of 5 h after oral administration of FR140423 (1–100 mg/kg) or indomethacin (1–32 mg/kg), the animals were killed with 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).

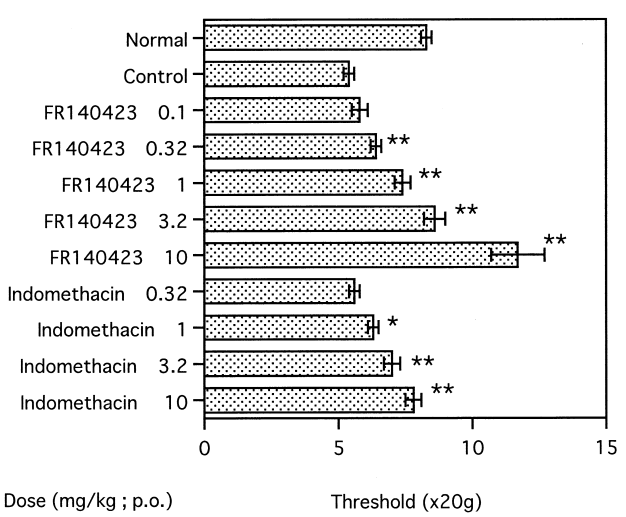

Fig. 5. Analgesic effect of FR140423 on yeast-induced hyperalgesia in rats. Brewer's yeast (5 mg/paw) was injected into the planter surface of the right paw. Drugs were given orally 2 h after yeast injection. The weight (g) applied to the yeast-injected paw that elicited pain responses such as struggling and squeaking was measured 3 h after yeast injection as the mechanical pain threshold. A cut-off value of 300 g was used to prevent damage to the paw. Significantly different from the control, $* P < 0.05$, $** P < 0.01$. $n = 5$. Values are means ± S.E.M.

2.7. Yeast-induced hyperalgesia

The yeast-induced hyperalgesia model was used as previously described (Randall and Selitto, 1957). Male Sprague–Dawley rats were fasted for 16 h before the experiment. Hyperalgesia was induced in rats by intradermal injection into the planter surface of the right hind paw of 0.1 ml of a 5% suspension of brewer's yeast (Asahi Beer Pharmaceutical, Tokyo, Japan) in 0.5% methylcellulose. A total of 2 h later, FR140423 (0.1–10 mg/kg), indomethacin (0.32–10 mg/kg) or morphine (0.1, 1 mg/kg), suspended and diluted in 0.5% methylcellulose, was administered orally, and pain thresholds in yeast-injected paw and normal non-injected paw were measured 3 h after yeast injection by using the Analgesy-Meter 7200 (Ugo Basile, Varese, Italy) with a pencil-shaped Teflon paw-presser; pressure was gradually applied to the hind paw. The end point was reached when the rat struggled and squeaked. Subcutaneous (s.c.) injection of naloxone HCl (Sigma) was given 2.5 h after yeast injection.

2.8. Tail-flick test in rats

Male Sprague–Dawley rats were fasted for 16 h and treated orally with drugs. Nociceptive thresholds were determined by using an automated Tail-flick Unit 7360 (Ugo Basile)(D'Amour and Smith, 1941). Radiant heat was adjusted to attain a mean baseline of 4–5 s in controls, and heat was automatically terminated at 15 s to avoid excessive tissue damage. After oral administration of FR140423 (3.2–100 mg/kg) or morphine (3.2, 10 mg/kg), suspended and diluted in 0.5% methylcellulose, the nociceptive responses in the tail-flick test were measured every 30 min for 120 min. Naloxone HCl (s.c.) was injected immediately before treatment of drugs.

2.9. Statistical analysis

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 their 95% confidence limits (95% C.L.) were calculated from the dose-percent inhibition relations by computer using log–linear regression analysis (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Effects of FR140423 on human recombinant cyclooxygenase-1 and cyclooxygenase-2 enzymes in vitro

Fig. 2 shows the effects of FR140423 on the activity of recombinant human cyclooxygenase-1 and cyclooxygenase-2. IC_{50} values for FR140423 were 19 ± 9 and 0.13 ± 0.06 μM for cyclooxygenase-1 and cyclooxygenase-2, respectively, which suggests that FR140423 was selective for cyclooxygenase-2. IC_{50} values for the reference compound, indomethacin, for cyclooxygenase-1 and cyclooxygenase-2 were 0.21 ± 0.14 and 0.16 ± 0.05 μM, respectively.

3.2. Anti-inflammatory effect of FR140423 on carrageenin-induced rat paw edema

FR140423 dose dependently inhibited carrageenin-induced rat paw edema with an ED_{50} value (95% C.L.) of

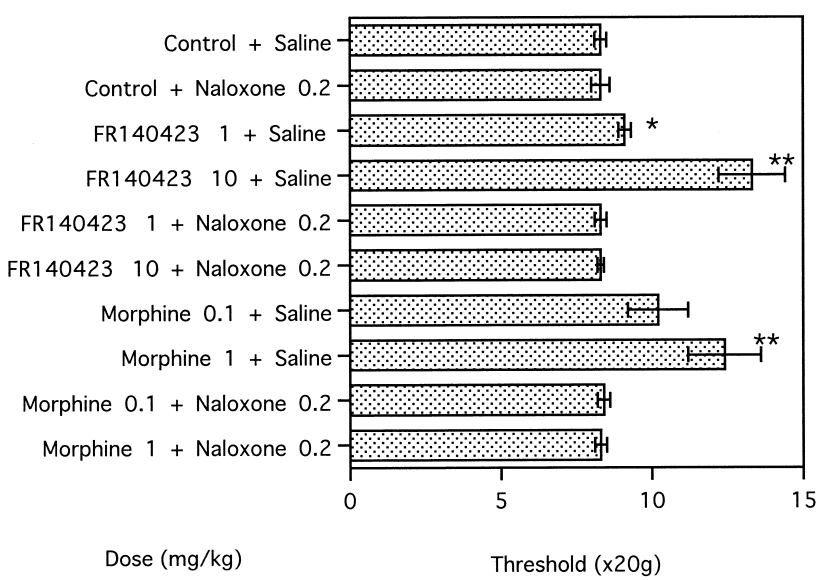

Fig. 6. Effect of naloxone on the FR140423 (p.o.)-induced analgesic effect in rat normal paws. Brewer's yeast (5 mg/paw) was injected into the planter surface of the right paw. Drugs were given orally 2 h after yeast injection, and pain thresholds in yeast non-injected left paw were measured 3 h after yeast injection by the paw pressure method. Naloxone (0.2 mg/kg s.c.) was injected 2.5 h after yeast injection. Significantly different from the control, $* P < 0.05$, $** P < 0.01$. $n = 5$. Values are means ± S.E.M.

2.6 (0.35–13) mg/kg, as shown in Fig. 3. The anti-inflammatory effect of FR140423 in this animal model was two times more potent than that of indomethacin.

3.3. Anti-inflammatory effect of FR140423 in adjuvant arthritic rats

Fig. 4 shows the anti-inflammatory effect of FR140423 in adjuvant arthritic rats. The edema in adjuvant-injected paws and uninjected paws on day 24 was 2.40 ± 0.20 and 1.28 ± 0.11 ml, respectively. Oral administration of FR140423 showed an anti-inflammatory effect in adjuvant arthritic rat paws in a dose-dependent manner. The effect of FR140423 was two- to three-fold more potent than that of indomethacin.

3.4. Gastric tolerability of drugs in rats

The gastric tolerability of FR140423 was evaluated after its acute oral administration to rats (Table 1). A single oral administration of FR140423 at doses between 1 and 100 mg/kg did not induce any mucosal lesions. By contrast, indomethacin induced marked gastric lesions in a dose-dependent manner with an UD_{50} value (95% C.L.) of 4.4 (0.93–13) mg/kg. FR140423 did not cause gastric side effects and had an apparent safety index (UD_{50}/ED_{50}), which was calculated with value of ED_{50} for carrageenin-induced edema and UD_{50}, in the stomach of more than 40.

3.5. Anti-hyperalgesic effect of FR140423 on yeast-induced hyperalgesia in rats

FR140423 showed a dose-dependent anti-hyperalgesic effect against yeast-induced paw hyperalgesia with an ED_{50} value (95% C.L.) of 0.46 (0.15–1.1) mg/kg (Fig. 5). Indomethacin also showed a dose-dependent anti-hyperalgesic effect in the inflamed paw with an ED_{50} value (95% C.L.) of 2.3 (0.60–12) mg/kg. Interestingly, unlike indomethacin, FR140423 caused a dose-dependent increase in the pain threshold in normal rat paws. The narcotic analgesic drug, morphine, had the same effect as FR140423 in normal rat paws, and both effects induced by morphine and FR140423 were abolished by pretreatment of the animals with the μ-opioid receptor antagonist naloxone (0.2 mg/kg s.c.) (Fig. 6).

3.6. Analgesic effect of FR140423 in tail-flick test in rats

The analgesic effect of FR140423 was further evaluated using an NSAID-insensitive nociceptive model, the tail-flick test in rats. Both drugs, FR140423 and morphine, showed a dose-dependent analgesic effect in the tail-flick test (Fig. 7), and these analgesic effects were abolished by pretreatment of the animals with naloxone (0.2 mg/kg s.c.) (Fig. 8). Indomethacin (3.2–32 mg/kg p.o.) did not show an analgesic effect in this assay (data not shown).

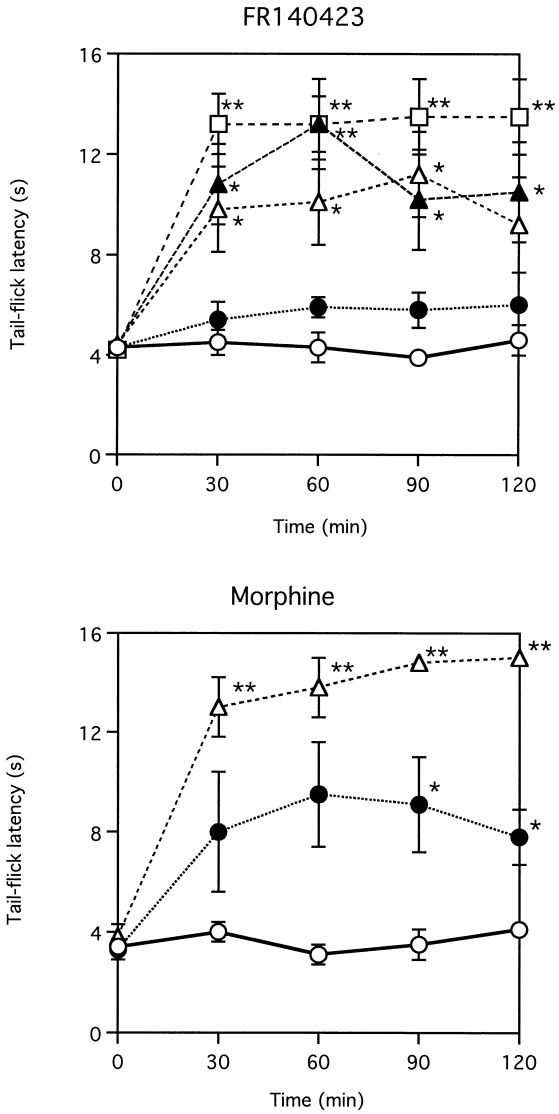

Fig. 7. Time course of antinociceptive effect of FR140423 in the tail-flick test in rats. FR140423 and morphine at 3.2 (closed circle), 10 (open triangle), 32 (closed triangle) and 100 (open square) mg/kg were administered p.o. at 0 time. The nociceptive responses in the tail-flick test were measured every 30 min for 120 min. A cut-off value of 15 s was used to avoid excessive tissue damage. Significantly different from the control (open circle), $*\ P < 0.05$, $**\ P < 0.01$. $n = 5$. Values are means ± S.E.M.

4. Discussion

It is well-known that the main mechanism of action of NSAIDs, which are widely used in rheumatoid arthritis as anti-inflammatory, anti-pyretic and analgesic drugs, is the inhibition of prostaglandin formation via the cyclooxygenase pathway. However, the general side effects of NSAIDs, ulcer induction and renal failure, are also considered to be the result of cyclooxygenase inhibition (Cryer and Feldman, 1992). Recently, two isoforms of cyclooxygenase, cyclooxygenase-1 and cyclooxygenase-2, have

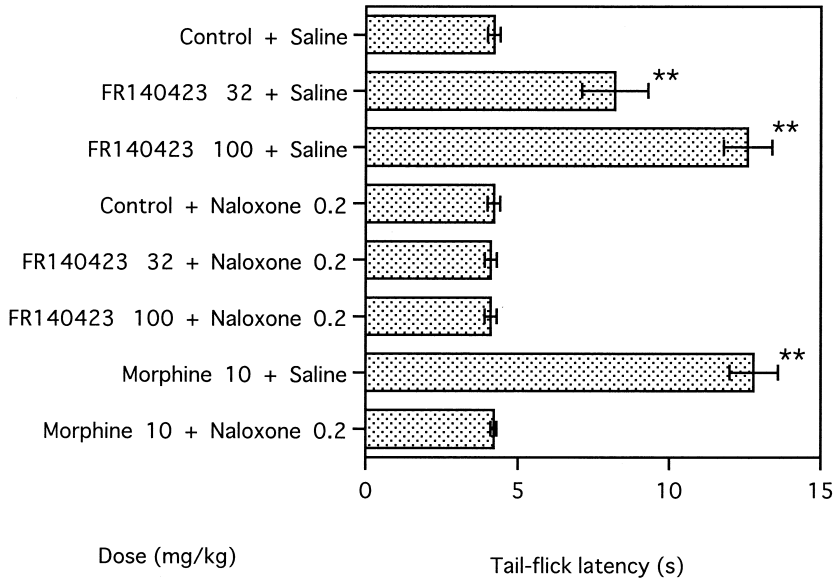

Fig. 8. Effect of naloxone on FR140423 (p.o.)-induced antinociception in the tail-flick test in rats. Data indicate the tail-flick latency 60 min after dosing. Naloxone (0.2 mg/kg s.c.) was injected immediately before treatment with the drugs. Significantly different from the control, $**P < 0.01$. $n = 5$. Values are means ± S.E.M.

been identified. Constitutive cyclooxygenase-1 is detected in most normal tissues and is involved in the maintenance of essential physiological functions such as platelet aggregation, cytoprotection in the stomach and normal kidney function. Cyclooxygenase-2 is an inducible isozyme that responds to inflammatory stimuli and plays an important role in the initiation and maintenance of various inflammatory conditions. Therefore, selective cyclooxygenase-2 inhibitors are expected to be new NSAIDs with reduced side effects. Recently, it has been reported that L-745,337 (5-methanesulfonamido-6-(2,4-difluorothiophenyl)-1-indanone), SC-58125 (1-[(4-methylsulfonyl)phenyl]-3-trifluorophenyl)pyrazole) and NS-398 (*N*-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide) are compounds in this category of new drugs (Futaki et al., 1993; Futaki et al., 1994; Seibert et al., 1994; Chan et al., 1995). A novel recently synthesized compound, FR140423, is 150 times more selective for cyclooxygenase-2 than for cyclooxygenase-1 in a human recombinant cyclooxygenase assay, in contrast to conventional NSAIDs, such as indomethacin, which inhibit both cyclooxygenase-1 and cyclooxygenase-2 with approximately equal potency. As expected, oral administration of indomethacin caused gastric mucosal damage at a low dose ($UD_{50} = 4.4$ mg/kg), whereas oral administration of the selective cyclooxygenase-2 inhibitor, FR140423, resulted in no visible gastric mucosal lesions in an acute ulcergenic assay at doses up to 100 mg/kg. The strong ulcergenic effect of indomethacin might be the result of its cyclooxygenase-1 inhibitory activity.

A good correlation between prostaglandin E_2 production and induction of cyclooxygenase-2 mRNA and its protein in inflamed paws has been reported in the carrageenin-induced rat paw edema model (acute inflammation) and in the rat adjuvant arthritis model (chronic inflammation) (Seibert et al., 1994; Anderson et al., 1996; Buritova et al., 1996). FR140423 showed potent anti-inflammatory effects in these models of inflammation. Other well-known selective cyclooxygenase-2 inhibitors, L-745,337, SC-58125 and NS-398, have been reported to give similar results (Futaki et al., 1993; Seibert et al., 1994; Chan et al., 1995). These results suggest that cyclooxygenase-2 but not cyclooxygenase-1 plays a major role in inflammatory responses.

Next, we estimated the analgesic effect of FR140423 using the yeast-induced hyperalgesic model. Several kinds of experimental hyperalgesic animal models have been reported and the involvement of cyclooxygenase in these models has been extensively studied. Opas et al. (1987) reported that the hyperalgesia induced in the rat paw after intraplantar injection of brewer's yeast was associated with the formation of cyclooxygenase products, prostaglandins. It is suggested that synthesized prostaglandins sensitize pain receptors in peripheral sites of inflammation, and induce the onset of hyperalgesia. However, the contribution of cyclooxygenase isozymes (cyclooxygenase-1 and cyclooxygenase-2) to hyperalgesia is still poorly understood. FR140423, a selective cyclooxygenase-2 inhibitor, showed an anti-hyperalgesic effect in this model, as does another selective cyclooxygenase-2 inhibitor, NS-398 (Futaki et al., 1993). This suggests that cyclooxygenase-2, but not cyclooxygenase-1, has an important role in the yeast-induced hyperalgesic model. While the effect of FR140423 on cyclooxygenase-2 activity was equal to that of indomethacin, the effect of FR140423 in the yeast-induced hyperalgesic model was five times more potent than that of indomethacin (Tsuji et al., 1997). One explanation

for this finding is to assume that FR140423 has another mechanism of action in addition to selective cyclooxygenase-2 inhibition.

Interestingly, unlike indomethacin, FR140423 increased the pain threshold not only in the inflamed paw but also in the normal non-inflamed paw. This effect was completely antagonized by the specific μ-opioid receptor antagonist naloxone. Furthermore, FR140423 showed an analgesic effect in the tail-flick method, which is generally used for testing centrally acting analgesic drugs, such as morphine. Indomethacin, which inhibits cyclooxygenase in inflamed paws, did not show any analgesic effect in the tail-flick test. This suggests that cyclooxygenase does not play an important role in the pain response in the tail-flick test. The analgesic effect of FR140423 in this tail-flick model was also antagonized by pretreatment of the animals with naloxone. This result suggests that this kind of analgesic effect of FR140423 is mediated by activation of μ-opioid receptors. Although further studies are required to clarify the precise mechanism of the analgesic effect of FR140423, it is clear that FR140423 has an analgesic effect mediated by opioid receptors in addition to the inhibition of prostaglandin formation as a result of its inhibition of cyclooxygenase-2 activity. This suggests that FR140423 is a unique compound with a pharmacological profile different from that of conventional NSAIDs such as indomethacin and the newly discovered selective cyclooxygenase-2 inhibitors such as L-745,337.

In conclusion, FR140423, which causes selective inhibition of cyclooxygenase-2, is a potent NSAID without gastrointestinal side effects and is a unique compound having a morphine-like central analgesic effect.

References

Anderson, G.D., Hauser, S.D., McGarity, K.L., Bremer, M.E., Isakson, P.C., Gregory, S.A., 1996. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J. Clin. Invest. 97, 2672–2679.

Buritova, J., Chapman, V., Honore, P., Besson, J., 1996. Selective cyclooxygenase-2 inhibition reduces carrageenan edema and associated spinal c-*Fos* expression in the rat. Brain Res. 715, 217–220.

Cao, C., Matsumura, K., Yamagata, K., Watanabe, Y., 1996. Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1β: a possible site of prostaglandin synthesis responsible for fever. Brain Res. 733, 263–272.

Chan, C., Boyce, S., Brideau, C., Ford-Hutchinson, A.W., Gordon, R., Guay, D., Hill, R.G., Li, C., Mancini, J., Penneton, M., Prasit, P., Rasori, R., Riendeau, D., Roy, P., Tagari, P., Vickers, P., Wong, E., Rodger, I.W., 1995. Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745,337: a novel non-steroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and non-human primate stomach. J. Pharmacol. Exp. Ther. 274, 1531–1537.

Cryer, B., Feldman, M., 1992. Effects of non-steroidal anti-inflammatory drugs on endogenous gastrointestinal prostaglandins and therapeutic strategies for prevention and treatment of non-steroidal anti-inflammatory drug-induced damage. Arch. Int. Med. 152, 1145–1155.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Day, R.O., 1988. Mode of action of non-steroidal anti-inflammatory drugs. Med. J. Aust. 148, 195–199.

Fernandez, A.G., Salcedo, C., Palacios, J.M., 1995. Aspirin, salicylate and gastrointestinal injury. Nat. Med. 1, 602–603.

Futaki, N., Yoshikawa, K., Hamasaka, Y., Arai, I., Higuchi, S., Iizuka, H., Otomo, S., 1993. NS-398, a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects, which causes minimal stomach lesions. Gen. Pharmacol. 24, 105–110.

Futaki, N., Takahashi, S., Yokoyama, M., Arai, I., Higuchi, S., Otomo, S., 1994. NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. Prostaglandins 47, 55–59.

Hla, T., Neilson, K., 1992. Human cyclooxygenase-2 cDNA. Proc. Natl. Acad. Sci. USA 89, 7384–7388.

Lichtenberger, L.M., Wang, Z., Romero, J.J., Ulloa, C., Perez, J.C., Giraud, M., Barreto, J.C., 1995. Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. Nat. Med. 1, 154–158.

Litchfield, J.T. Jr., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Meade, E.A., Smith, W.L., DeWitt, D.L., 1993. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J. Biol. Chem. 268, 6610–6614.

Newbould, B.B., 1963. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br. J. Pharmacol. 21, 127–136.

Opas, E.E., Dallob, A., Herold, E., Luell, S., Humes, J.L., 1987. Pharmacological modulation of eicosanoid levels and hyperalgesia in yeast-induced inflammation. Biochem. Pharmacol. 36, 547–551.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. 111, 409–419.

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L., Isakson, P., 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation and pain. Proc. Natl. Acad. Sci. USA 91, 12013–12017.

Tsuji, K., Konishi, N., Spears, G.W., Ogino, T., Nakamura, K., Tojo, T., Ochi, T., Shimojo, F., Senoh, H., Matsuo, M., 1997. Studies on anti-inflammatory agents: V. Synthesis and pharmacological properties of 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole and related compounds. Chem. Pharm. Bull. 45, 1475–1481.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New Biol. 231, 232–235.

Walz, D.T., DiMartino, M.J., Kuch, J.H., Zuccarello, W., 1971. Adjuvant-induced arthritis in rats: temporal relationship of physiological, biochemical, and hematological parameters. Proc. Soc. Exp. Biol. Med. 136, 907–910.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Xie, W., Robertson, D.L., Simmons, D.L., 1992. Mitogen-inducible prostaglandin G/H synthase: a new target for non-steroidal anti-inflammatory drugs. Drug Dev. Res. 25, 249–265.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.