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Preclinical pharmacology profile of CS-706, a novel cyclooxygenase-2 selective inhibitor, with potent antinociceptive and anti-inflammatory effects

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Abstract

We report here the preclinical anti-inflammatory profile of CS-706 [2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-1*H*-pyrrole], a novel cyclooxygenase-2 (COX-2) selective inhibitor. CS-706 selectively inhibited COX-2 in a human whole blood assay with an IC_{50} of 0.31 μM, compared with an IC_{50} of 2.2 μM for COX-1. The selectivity ratio of CS-706 was higher than those of the conventional non-steroidal anti-inflammatory drugs naproxen, indomethacin, and Diclofenac–Na, whereas it was lower than those of rofecoxib, valdecoxib and etoricoxib. It was similar to that of celecoxib. The pharmacokinetic profile of CS-706 showed rapid absorption and dose-proportional exposure after oral administration to rats. CS-706 inhibited prostaglandin E_2 production in inflamed tissue induced by yeast-injection in rats with potency similar to that of indomethacin. However, it inhibited gastric mucosal prostaglandin E_2 production in normal rats weakly compared with indomethacin. CS-706 ameliorated both yeast-induced inflammatory acute pain (ED_{50}=0.0090 mg/kg) and adjuvant-induced chronic arthritic pain (ED_{50}=0.30 mg/kg) in rats. CS-706 showed more potent antinociceptive activity than celecoxib and rofecoxib in these models. In an adjuvant-induced arthritic model in rats, CS-706 suppressed foot swelling prophylactically with an ID_{50} of 0.10 mg/kg/day, and decreased foot swelling in the established arthritis therapeutically in a dose range of 0.040 to 1.0 mg/kg/day. Single administration of up to 100 mg/kg of CS-706 induced no significant gastric lesions in rats. In conclusion, CS-706 is a COX-2-selective inhibitor with a potent antinociceptive and anti-inflammatory activity and a gastric safety profile.

Keywords: Cyclooxygenase-2; COX-2-selective inhibitor; CS-706 [2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-1*H*-pyrrole]; Inflammation; Nociception; Non-steroidal anti-inflammatory drug

1. Introduction

Conventional non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of rheumatoid arthritis, osteoarthritis and pain (dental pain, post-operative pain, etc.). However, it is also known that side effects on the gastrointestinal tract, such as gastric lesions and intestinal ulcers, occur frequently (Langman et al., 1994). The mechanism of action of NSAIDs is the inhibition of cyclooxygenase (COX), the key enzyme of prostaglandin biosynthesis. This enzyme has been found to exist in two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in platelets and normal cells in the gastrointestinal tract, kidneys, etc. to maintain homeostasis, whereas COX-2 is specifically expressed in the inflammatory cells and is involved in acute and chronic inflammatory responses (Hla and Nelson, 1992; Seibert et al., 1994). It is thought that the traditional NSAIDs inhibit both COX-1 and COX-2 activities and that COX-1 inhibition causes the side effects on the gastrointestinal tract, etc. Therefore, COX-2-selective inhibitors were expected to be the next generation NSAIDs with fewer side effects on the gastrointestinal tract (Masferrer et al., 1994; Seibert et al., 1994).

Over the past years, several COX-2-selective inhibitors have appeared on the market. These include celecoxib, rofecoxib, valdecoxib, parecoxib, etoricoxib and lumiracoxib. All have shown a reduced risk of inducing gastroduodenal injury, compared with

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traditional NSAIDs (Singh et al., 2006; Bombardier et al., 2000; Goldstein et al., 2004; Harris et al., 2004; Schnitzer et al., 2004). In addition, COX-2-selective inhibitors are found to be efficacious for a broad array of indications, including acute pain/analgesia, primary dysmenorrhea, rheumatoid arthritis and osteoarthritis. However, in 2004, rofecoxib was found to be related to increased thrombotic cardiovascular events and was withdrawn from the market (Food and Drug Administration, 2004; Bresalier et al., 2005). Valdecoxib was also withdrawn, due at least in part to cardiotoxicity in high-risk cardiac patients (Nussmeier et al., 2005). Cardiovascular risk elevation was also reported in prophylactic trials of celecoxib and naproxen on colon-rectal cancers and Alzheimer's disease, respectively (Solomon et al., 2005). A subsequent systematical review of the evidence showed that the cardiovascular risks of COX-2-selective inhibitors appear to be dependant on not only drug class but also on the individual drugs, and that the risk with celecoxib is less than that with rofecoxib, and that is comparable to most traditional NSAIDs (Graham et al., 2005; Brophy, 2005; Jones, 2005; McGettigan and Henry, 2006; Solomon et al., 2006). Celecoxib is now the only COX-2 inhibitor on the market in the US.

However, the clinical effect of celecoxib is less potent than those of naproxen or rofecoxib particularly in terms of analgesic activity (Malmstrom et al., 1999). Thus, a new generation of COX-2 inhibitors with greater efficacy is required. On the other hand, rofecoxib requires caution regarding the risk of cardiovascular events. The cause of the higher cardiovascular risk of rofecoxib compared with celecoxib is not fully understood, but one of the reasons is considered to be its high COX-2 selectivity (Brophy, 2005; Jones, 2005). On the contrary, both celecoxib and rofecoxib have shown reduction of gastrointestinal risk at clinical doses (Singh et al., 2006; Bombardier et al., 2000), suggesting that the high selectivity of rofecoxib is not necessary for avoiding gastrointestinal toxicity. Consequently, it might be plausible to speculate that moderate COX-2 selectivity minimizes both gastrointestinal events and the risk of cardiovascular events. Therefore, we have screened a variety of compounds in order to obtain an agent with more potent anti-inflammatory and antinociceptive effects than celecoxib and with moderate COX-2 selectivity comparable to that of celecoxib. We have discovered CS-706 [2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-1*H*-pyrrole]. Early phase II clinical trials of this agent have been completed in the United States, and its efficacy and safety have been demonstrated (Moberly et al., 2007a,b).

In the present study, we investigate the preclinical pharmacological profile of CS-706 with regard to *in vitro* COX-2 selectivity using a human whole blood assay, the pharmacokinetic profile, the *in vivo* tissue selectivity of prostaglandin content reduction in rats, and the anti-inflammatory and antinociceptive activities in a variety of inflammatory models in rats.

2. Materials and methods

2.1. Animals

Wistar–Imamichi rats were purchased at the age of 4 to 5 weeks from the Imamichi Institute for Animal Reproduction (Ibaraki, Japan) and Lewis rats were purchased at the age of 5 to 9 weeks from Charles River Japan Inc. (Kanagawa, Japan). The animals were housed in an air-conditioned room with controlled temperature (23 ± 2 °C) and humidity (55 ± 5%), and with a 12-h light/dark cycle (light from 7:00 to 19:00). They were fed and given water *ad libitum* throughout the experimental period, unless otherwise noted. They were acclimated for about 1 week before use. All the animal care and experiments were carried out under the standard operation procedures approved by the Sankyo Institutional Animal Care and Use Committee.

2.2. Reagents

CS-706 [2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-1*H*-pyrrole], celecoxib, rofecoxib, valdecoxib, etoricoxib, L-745337 [5-methanesulphonamido-6-(2,4-difluorothio-phenyl)-1-indanone], NS-398[N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulfonamide], nimesulide, meloxicam, nabumeton, naproxen, Diclofenac–Na and indomethacin were all prepared at the Medicinal Chemistry Research Laboratories, Sankyo Co. Ltd. (Tokyo, Japan). The structural formula of CS-706 is shown in Fig. 1. Arachidonic acid, glutathione (reduced form), epinephrine, and yeast (YBD) were obtained from Sigma-Aldrich (St. Louis, MO); lipopolysaccharide (LPS, B E. coli 026:B6) and *Mycobacterium butyricum* were from BD Diagnostic Systems (Sparks, MD); liquid paraffin was from Wako (Osaka, Japan); A23187 (Ca^{2+} ionophore) was from Calbiochem-Novabiochem Corp. (San Diego, CA); prostaglandin E_2 and thromboxane B_2 enzyme immunoassay (EIA) kits were from Cayman Chemical Co. (Ann Arbor, MI); tragacanth powder was from Nippon Funmatsu Yakuhin & Co., Ltd. (Ohsaka, Japan). For the *in vitro* assays, the test compounds were freshly dissolved in dimethyl sulfoxide (DMSO) before use. For the animal experiments, the test compounds were suspended in 0.5% tragacanth suspension before use, and a volume of 5 ml/kg was administered orally to animals unless otherwise noted. The vehicle (0.5% tragacanth suspension) was administered to the control group (5 ml/kg).

2.3. In vitro assays

2.3.1. Human whole blood COX-1 and COX-2 assay

The assays were performed according to the method described previously (Young et al., 1996). Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. For the COX-1 assay, blood aliquots of

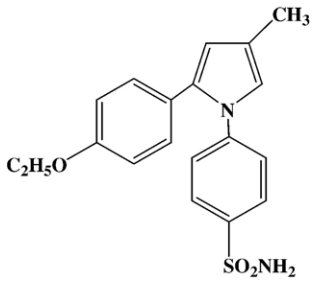

Fig. 1. Chemical structure of CS-706.

500 μl were mixed with 2 μl of either various concentrations of the test compound (dissolved in DMSO) or DMSO (control) in microtubes and preincubated at 37 °C for 4 h in a CO_2 incubator. The blood was then mixed with 2 μl of 7.5 mM A23187 (dissolved in DMSO, at a final concentration of 30 μM) and incubated for 1 h under the same conditions, immediately chilled at 4 °C and then centrifuged at 15,300 ×*g* for 5 min. The plasma was stored at −20 °C until use. A blank assay was carried out by mixing blood with 4 μl DMSO instead of the test compound and A23187. Concentrations of thromboxane B_2 in the plasma were determined as COX-1 dependent production by commercially available EIA kits. For the COX-2 assay, blood aliquots of 500 μl were mixed with 2 μl of either various concentrations of the test compound (dissolved in DMSO) or DMSO (control) and with 10 μl of 0.5 mg/ml LPS (dissolved in saline, at a final concentration of 10 μg/ml) in microtubes. The blood was incubated at 37 °C for 5 h in a CO_2 incubator, immediately chilled at 4 °C and then centrifuged at 15,300 ×g for 5 min. The plasma was stored at-20 °C until use. A blank assay was carried out by mixing blood with 2 μl of DMSO and 10 μl of saline instead of the test compound and LPS. Concentrations of thromboxane B_2 in the plasma were determined as COX-2 dependent production by commercially available EIA kits.

2.3.2. Human recombinant COX-1 and COX-2 assay

pcDL-SRα296 carrying human COX-1 and -2 cDNAs were supplied by Dr. Tanzawa (Biological Research Laboratories) and Dr. Ogata (Lead Discovery Research Laboratories) of Sankyo Co., Ltd. and transiently transfected into subconfluent COS cells by an electroporation method, and the cells were incubated for 66 h in cell culture plates in a similar fashion to those described previously (Hla and Neilson, 1992; Fletcher et al., 1992; Takebe et al., 1988). The preparation of COS cell microsomes and COX-1 and-2 enzyme assays were conducted according to the method described previously (Matsuda et al., 1984). Briefly, enzyme activity was assayed using a reaction mixture of a total volume of 100 μl/well, which contained 100 mM Tris–HCl buffer (pH 7.6), 2 mM glutathione, 1 mM epinephrine, 20 μg protein of microsomes of COS cells expressing COX-1 or COX-2, various concentrations of CS-706 (dissolved in DMSO) or DMSO (control), and 10 μM arachidonic acid (dissolved in ethanol) in a 96-well plastic plate. The final concentrations of DMSO and ethanol in the reaction mixture were 2% and 2.5%, respectively. The microsomes were preincubated with the compound or DMSO at 37 °C for 15 min. The reaction was started by adding arachidonic acid and the mixture was incubated at 37 °C for 30 min. The reaction was then stopped by adding 15 μl/well of ice-cold 0.2 M HCl. The mixture was maintained at 4 °C for 5 min and then neutralized by adding 15 μl/well of 0.2 M NaOH. A blank assay was carried out by adding a mixture with DMSO and ethanol instead of the test compound and arachidonic acid. Concentrations of prostaglandin E_2 in the reaction mixture were determined by commercially available EIA kits.

2.3.3. Data analysis

The percent inhibitions of both the whole blood and recombinant COX activities by the compound were calculated by a following equation. Percent inhibition = {1 − (concentration of prostaglandin in the reaction mixture of the test compound − concentration of prostaglandin in the reaction mixture of blank)/(concentration of prostaglandin in the reaction mixture of control − concentration of prostaglandin in the reaction mixture of blank)} × 100. IC_{50} values were calculated based on linear regression lines obtained from the percent inhibitions and the logarithmic values of the concentrations by the least squares method. The 95% confidence intervals were calculated by Fieller's theorem.

2.4. Pharmacokinetics of CS-706

Female Lewis rats (170–200 g), fasted overnight before dosing and through 8 h post-dose, were orally administered with CS-706 in suspension at doses of 0.5, 1.0, 2.5 and 5 mg/kg. Blood was collected from the jugular vein at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post-dose and centrifuged (19,000 ×*g* for 3 min) to obtain the plasma. Concentrations of CS-706 in the plasma were determined by high performance liquid chromatography (HPLC) with UV detection. An aliquot of plasma (100 μl) was added to the internal standard (100 μl of 0.5 μg/ml n-Heptyl 4-hydroxybenzoate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) solution in CH_3CN) and CH_3CN (100 μl), mixed by a vortex mixer and centrifuged (19,000 ×g for 5 min) to prepare the samples for HPLC. HPLC analysis was carried out on an LC-10A instrument with a UV detector (Shimadzu Corp., Kyoto, Japan). The mobile phase (CH_3CN/0.1% (v/v) acetic acid = 3/2) was delivered isocratically at a flow rate of 1 ml/min. Samples (100 μl) were injected onto a YMC-Pack ODS-A A-312 column (150 × 6.0 mm I.D., S-5 μm, YMC Co., Ltd.) at 40 °C. UV detection was achieved at a wavelength of 262 nm. The pharmacokinetic parameters of the plasma CS-706 were calculated by non-compartmental analysis using WinNonlin Professional (Ver. 4.0.1., Pharsight Corp.).

2.5. In vivo assay of tissue selective inhibition of prostaglandin contents

2.5.1. Prostaglandin E_2 contents in stomach

Assays of prostaglandin E_2 contents in stomach were conducted using the same procedure as that of the gastric mucosal lesion experiments in rats. A single dose of CS-706, indomethacin, as a reference agent, or the vehicle was administered orally to male Wistar–Imamichi rats (70–100 g) after overnight fasting. The rats were sacrificed 3.5 h after administration by exsanguinations from the carotid arteries. The stomachs were removed and dissected, followed by a wash with saline at 4 °C, and then snap frozen with a metal clamp pre-cooled with liquid nitrogen. Then, the mucosa was separated from the muscular coat, weighed and kept at −80 °C until the prostaglandin E_2 assay. The frozen tissues were cut into pieces in an ice-chilled solution consisting of 3.0 ml of methanol and 0.25 ml of 0.5 M potassium phosphate buffer (pH = 6.8), supplemented with 200 μM indomethacin and homogenized at 4 °C for 30 s with Polytron® homogenizer (Kinematica AG, Lucerne, Switzerland). The homogenate was then added to 2.5 ml of chloroform, mixed for 1 min with a vortex mixer, and centrifuged at 1560 ×g at 4 °C for 15 min. The resulting supernatant

fraction (1 ml) was collected and the organic solvent was evaporated under a stream of nitrogen. The dried residue was dissolved in 0.05 ml of the buffer provided by prostaglandin E_2 EIA kits, and prostaglandin E_2 was assayed with prostaglandin E_2 EIA kits.

2.5.2. Prostaglandin E_2 contents in inflamed tissue

Assays of prostaglandin E_2 contents in inflamed tissue were conducted in an inflammation model in rats. Inflammation was induced in the feet of the rats by a method similar to that used to test the antinociceptive effect of the compounds on yeast-induced acute inflammatory pain. Male Wistar–Imamichi rats (60–80 g) were subjected to a subcutaneous injection of 0.1 ml of a 20% suspension of dead Brewer's yeast into the right hind paw after overnight fasting. In a preliminary experiment, the time course of prostaglandin E_2 level in the right hind feet after the yeast-injection was investigated. The result indicated that the prostaglandin E_2 level was submaximal at 8 h after the induction of inflammation. Therefore, a single dose of CS-706, indomethacin or the vehicle was administered to the animals 6 h after yeast-injection and the rats were sacrificed 2 h after administration (8 h after induction of inflammation) by CO_2 inhalation and the right hind feet were immediately removed and weighed. Groups which were not injected with yeast were regarded as the normal groups. The feet were snap frozen with a metal clamp pre-cooled with liquid nitrogen, and stored at −80 °C until use. The feet were then crushed with a Cryo-press® (Microtec Co., Ltd., Chiba, Japan) in the presence of liquid nitrogen, and homogenized with a Polytron® homogenizer at 4 °C twice for 60 s in 7 ml PBS supplemented with 10 mM EDTA and 100 μM indomethacin. The homogenate was centrifuged at 1560 ×g at 4 °C for 15 min, and the supernatant fraction was stored at-20 °C until the prostaglandin E_2 assay with EIA kits.

2.5.3. Data analysis

In the gastric mucosal tissue assay, the percent inhibition of prostaglandin E_2 content by a compound was calculated by the following equation: {1 − (prostaglandin E_2 content of a compound-treated group)/(prostaglandin E_2 content of a control group)} × 100. In the inflamed tissue assay, the percent inhibition of prostaglandin E_2 content by a compound was calculated by the following equation: {1 − (prostaglandin E_2 content of a compound-treated rat − prostaglandin E_2 content of a normal group)/(prostaglandin E_2 content of a control group − prostaglandin E_2 content of a normal group)} × 100. ID_{50} values were calculated based on linear regression lines obtained from the percent inhibitions and the logarithmic values of the doses by the least squares method. The 95% confidence intervals were calculated by Fieller's theorem.

2.6. Antinociceptive effect on in vivo models

2.6.1. Yeast-induced acute inflammatory pain in rats (Randall–Selitto method)

Experiments were carried out according to the method described previously (Randall and Selitto, 1957) with modifications (Winter and Flataker, 1965). Male Wistar–Imamichi rats (60–80 g) were subjected to a subcutaneous injection of 0.1 ml of a 20% suspension of dead Brewer's yeast into the right hind paw after overnight fasting. A hypernociceptive state was produced by applying 250 g of pressure to the inflamed paw twice at 2 and 4 h after the injection by a balance pressure device (Ugo-Basile, Italy). A constant increase in pressure was applied to the inflamed paw 4.5 h after the yeast-injection and the pain threshold was determined by measuring the pressure in g at the time when the animal began to cry and struggle. Animals with a pain threshold of less than 150 g were selected for the antinociceptive assays, and were immediately administered orally with test compounds or the vehicle, and the pain thresholds at 0.5, 1 and 2 h after the dosing were measured. When the pain threshold of the animal at any of the time points of 0.5, 1 and 2 h was 2 times higher than the mean pain threshold at the corresponding time points in the vehicle-administered group (control), the animal was defined as antinociceptive-positive.

2.6.2. Chronic inflammatory pain in adjuvant-induced arthritis in rats

Experiments were carried out according to the method described previously (Kuzuna and Kawai, 1975) with slight modifications. Briefly, an adjuvant was prepared by suspending heat-killed dried *Mycobacterium butyricum* in dry-sterilized liquid paraffin to make a 2.0 mg/ml suspension and sonicated with Sonifier® Cell Disruptor 200 (Branson Ultrasonic, Danbury, CT). The adjuvant (100 μg/0.05 ml/paw) was injected intradermally into the heel of the right hind footpad of male Lewis rats (140–160 g) on Day 0. Rats with well-established arthritis were fasted overnight on Day 17, and the pain response of the animals was examined by gently flexing the tarso-tibial joint of the uninjected foot 5 times at intervals of 4 to 5 s on Day 18. Animals squeaking at every flexion were defined as pain-positive. The pain-positive rats were then randomly divided into groups and were orally administered with test compounds or the vehicle. The pain-response was examined at 0.5, 1, 2 and 4 h after administration by flexing the uninjected foot 5 times in the same way, and the number of times the rat squeaked was recorded as the pain score. An animal was defined as antinociceptive-positive when its pain score contained "0" at least once within 2 h at 0.5, 1 or 2 h after administration.

2.6.3. Data analysis

ED_{50} values (doses producing 50% effectiveness) and the 95% confidence intervals were calculated by the Probit method based on the incidence of antinociceptive-positive animals as a function of the doses of the compound.

2.7. Anti-swelling effect on in vivo models

2.7.1. Prophylactic effect on adjuvant-induced arthritis in rats

Experiments were performed according to the method described previously (Winder et al., 1969) with slight modifications. Briefly, an adjuvant (100 μg *M. butyricum*/0.05 ml/paw) was prepared and injected intradermally in the heel of the right hind footpad of female Lewis rats (170–200 g) on Day 0, as described above. The test compounds or the vehicle were orally administered at a volume of

5 ml/kg once a day for 21 days, and the volume of the adjuvant-injected foot was measured on Days 3, 5, 7, 10, 13, 15, 18 and 21 using a plethysmometer (Ugo Basile, Italy).

2.7.2. Therapeutic effect on established adjuvant-induced arthritis in rats

Experiments were performed by a method similar to the one described above. Briefly, an adjuvant (100 μg *M. butyricum*/0.05 ml/paw) was prepared and injected intradermally in the heel of the right hind footpad of female Lewis rats (170–200 g) on Day 0. On Day 18, when the swelled foot volume almost reached a plateau in the majority of the animals, the animals with prominent swelling in the adjuvant-injected foot were selected. The animals were divided into groups so that the mean swelled foot volumes were equivalent. The test compounds or the vehicle were orally administered at a volume of 2 ml/kg twice daily from Day 18 to Day 24. The volume of the adjuvant-injected foot was measured on Days 18, 20, 23 and 25 using a plethysmometer.

2.7.3. Data analysis

The swelled foot volume was calculated by the following equation: (hind foot volume of an adjuvant-injected animal) − (mean hind foot volume of normal animals). In the prophylactic experiments, the percent inhibition of the swelled foot volume on Day 21 was calculated by the following equation: {1 − (swelled foot volume of a compound-treated animal on Day 21)/(mean swelled foot volume of the vehicle-treated animals on Day 21)} × 100. ID_{50} values were calculated based on linear regression lines obtained from the percent inhibitions and the logarithmic values of the doses by the least squares method. The 95% confidence intervals were calculated by Fieller's theorem. In the therapeutic experiments, the ratio of the swelled foot volume was first calculated as follows: (swelled foot volume of animals on Day 25)/(swelled foot volume of animals on Day 18) × 100, and the percent inhibition of the ratio of swelled foot volume was calculated against the vehicle-treated group.

Table 1
Inhibition of human whole blood COX-1 and COX-2 activity

	IC_{50} for COX-1 (μM) (95% CI)	IC_{50} for COX-2 (μM) (95% CI)	Selectivity ratio ($IC_{50,\ COX\text{-}1}$/$IC_{50,\ COX\text{-}2}$)
Nabumeton	23 (16–32)	190 (N.D.)	0.12
Naproxen	7.0 (5.8–8.4)	33 (17–94)	0.21
Indomethacin	0.14 (0.096–0.19)	0.64 (0.48–0.87)	0.22
Diclofenac–Na	0.076 (0.051–0.11)	0.079 (0.055–0.11)	0.96
Meloxicam	1.6 (0.89–2.6)	0.93 (0.69–1.4)	1.7
Nimesulide	9.0 (7.4–11)	1.7 (1.1–3.5)	5.3
CS-706	2.2 (1.3–3.4)	0.31 (0.15–0.63)	7.1
Celecoxib	8.3 (5.9–13)	0.93 (0.47–2.8)	8.9
NS-398	6.1 (4.6–7.7)	0.27 (0.13–0.87)	23
Rofecoxib	6.5 (3.0–11)	0.20 (0.15–0.26)	33
L-745337	150 (130–170)	4.0 (2.6–5.9)	38
Valdecoxib	28 (7.3–74)	0.60 (0.46–0.79)	47
Etoricoxib	49 (32–84)	0.35 (0.27–0.43)	140

For determination of COX-1 activity, human whole blood was pre-incubated with CS-706 or other test compounds at 37 °C for 4 h. The Ca^{2+} ionophore, A23187 (final, 30 μM) was added to the reaction mixture and the mixture was incubated for further 1 h. Thromboxane B_2 produced in plasma (COX-1 dependent production) was measured by EIA. For determination of COX-2 activity, human whole blood was incubated with CS-706 or other test compounds, and lipopolysaccharide (final, 10 μg/ml) for 5 h. Thromboxane B_2 produced in plasma (COX-2 dependent production) was measured by EIA. The results were the means of 3–6 experiments, each of which was carried out in duplicate or triplicate.
N.D.: not determined.

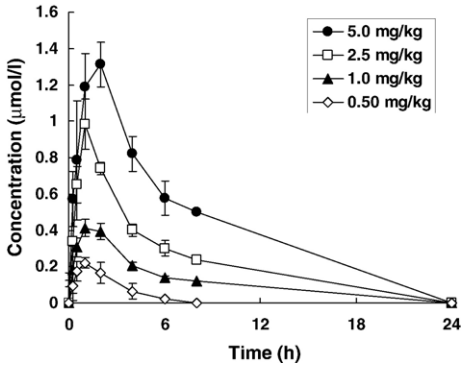

Fig. 2. Plasma concentrations of CS-706 after oral administration of CS-706 in rats. CS-706 was orally administered at doses of 0.50, 1.0, 2.5 and 5.0 mg/kg. Plasma samples were processed through a C_{18} reverse-phase HPLC column, and the plasma levels were determined by HPLC with UV detection. Each data point represents the mean ± S.D. of 3 animals.

2.8. Gastric mucosal lesion experiments in rats

2.8.1. Lesion formation

Gastric mucosal lesions by test compounds were studied according to the method described previously (Jahn and Adrian, 1969). A single dose of the test compounds or the vehicle was administered orally to male Wistar–Imamichi rats (70–100 g) after overnight fasting. The rats were sacrificed 3.5 h after administration by exposure to CO_2. The stomach was ligated at the esophagus with forceps, and approximately 10 ml of 0.5% formaldehyde solution was poured into the stomach from the duodenum and the stomach was removed. After immersing the stomach in 0.5% formaldehyde solution for 1–2 h, it was opened along the greater curvature. The mucosa was then examined for the presence of hemorrhage lesions under a microscope (6.3 × 10 magnification). Gastric mucosal lesion-inducing activity by a compound was

Table 2
Pharmacokinetic parameters of CS-706 in plasma after oral administration of CS-706 in rats

Dose (mg/kg)	t_{max} (h)	C_{max} (μmol/ml)	AUC (μmol·h/ml)	$t_{1/2}$ (h)
0.50	1.0 ± 0.00	0.22 ± 0.054	0.58 ± 0.32	N.D. [a]
1.0	1.3 ± 0.58	0.41 ± 0.053	1.9 ± 0.22	3.5 ± 0.44
2.5	1.0 ± 0.00	0.98 ± 0.10	3.8 ± 0.36	5.8 ± 2.1
5.0	1.7 ± 0.58	1.4 ± 0.11	6.6 ± 0.59	5.3 ± 2.9

Results are given as the mean ± S.D. of 3 animals. t_{max}, time of maximum concentration observed; C_{max}, maximum plasma concentration; AUC, area under the concentration-time curve up to the last quantifiable time; $t_{1/2}$, elimination terminal half-life.
[a] $t_{1/2}$ at 0.5 mg/kg was not determined because sufficient data about the elimination phase were not obtained.

determined by the method described previously (Hitchens et al., 1967). Dark brown hemorrhage lesions of more than 0.5 mm in length were measured and the total length was determined for the individual animals. When an animal had hemorrhage lesions with a total length of more than 2.0 mm, the animal was considered to be lesion positive. The incidence of positive animals was determined in each group.

2.8.2. Data analysis

UD_{50} values (doses producing 50% incidence) and the 95% confidence intervals were calculated by the Probit method based on the incidence of positive animals as a function of the doses of the compound.

3. Results

3.1. In vitro studies

3.1.1. Inhibition of human whole blood COX-1 and COX-2 activity

Inhibition of COX-1 and COX-2 activities and COX-2 selectivity for CS-706 were evaluated in human whole blood assays and compared with other COX-2 inhibitors and conventional NSAIDs

Human whole blood assays have been established to be the standard approach to gauge the COX selectivity of NSAIDs. All the compounds tested showed concentration-dependent inhibition of both COX-1/-2 activities, and IC_{50} values for each COX activity and the selectivity ratios of the compounds are summarized in Table 1.

CS-706 inhibited whole blood COX-1 and COX-2 activities with IC_{50} values of 2.2 μM and 0.31 μM, respectively. The COX-2 selectivity ratio ($IC_{50,\ COX\text{-}1}/IC_{50,\ COX\text{-}2}$) was 7.1-fold. IC_{50}s for COX-2 were somewhat similar among the other compounds except for nabumeton and naproxen, while those for COX-1 varied widely. The COX-2 selectivity of CS-706 was higher than those of nabumeton, naproxen, indomethacin, Diclofenac–Na, meloxicam and nimesulide, while it was lower than those of rofecoxib, valdecoxib and etoricoxib and others. It is most similar to that of celecoxib (8.9-fold) (Table 1).

3.1.2. Inhibition of human recombinant COX-1 and COX-2 activity

CS-706 was evaluated for its inhibitory activity of COX isoforms using human recombinant enzymes. CS-706 potently inhibited COX-2 enzyme activity with an IC_{50} of 0.087 μM (95% C.I. 0.061–0.12) whereas the compound inhibited COX-1 activity with an IC_{50} of 2.5 μM (95% C.I. 1.9–3.3). CS-706 had COX-2 selectivity of approximately 29-fold compared with COX-1 in this assay system.

3.2. Pharmacokinetics of CS-706

The pharmacokinetic profile was examined after a single oral administration of CS-706 to rats in the dose range of 0.5 to 5 mg/kg prior to the following *in vivo* pharmacology studies, and is shown in Fig. 2 and Table 2. CS-706 was rapidly absorbed after administration and the plasma concentration reached C_{max} (maximum plasma concentration) between 1 to 2 h (t_{max}, time of maximum concentration observed). The plasma concentrations then gradually declined with $t_{1/2}$ (elimination terminal half-life) of 3.5 to 5.8 h and fell below measurable limits (LLOQ, 0.025 μg/ml) at 24 h post-dose. C_{max} and AUC (the area under the plasma concentration-time curve up to the last quantifiable time) increased dose-proportionally in the dose range. Only the C_{max} at 5 mg/kg was a little lower than the extrapolated value with a

Table 3
Inhibition of prostaglandin E_2 contents in gastric mucosa and in inflamed tissue in rats

	Prostaglandin E_2 content in gastric mucosa				Prostaglandin E_2 content in inflamed tissue				Selectivity ratio
	Dose (mg/kg)	*n*	% inhibition	ID_{50} (mg/kg) (95% C.I.)	Dose (mg/kg)	*n*	% inhibition	ID_{50} (mg/kg) (95% C.I.)	($ID_{50,\ gastric\ mucosa}$/ $ID_{50,\ inflamed\ tissue}$)
CS-706	0.0	6			0.0	8			
	0.31	5	15		0.030	8	45		
	1.0	5	46	1.6	0.10	8	52	0.073	22
	3.0	5	70	(0.89–2.8)	0.30	8	56	(0.023–0.23)	
	10	6	78		1.0	8	76		
	30	4	86						
Indomethacin	0.0	11			0.0	8			
	0.031	11	3		0.10	8	31		
	0.10	11	23	0.23	0.30	8	65	0.20	1.2
	0.30	12	64	(0.18−0.31)	1.0	8	88	(0.15–0.26)	
	1.0	10	85		3.0	8	86		
	3.0	5	91						

For determining decrease in prostaglandin E_2 contents in gastric mucosa, a single oral dose of the test compounds or the vehicle was administered to male Wistar–Imamichi rats after overnight fasting. The rats were sacrificed 3.5 h after dosing and the prostaglandin E_2 contents in mucosa were measured by prostaglandin E_2 EIA kits. For determining decrease in prostaglandin E_2 contents in inflamed tissue, inflammation was induced by an injection of 20% dead Brewer's yeast into the right hind paw of male Wistar–Imamihi rats after overnight fasting. A single oral dose of the test compounds or the vehicle was administered 6 h later to the rats. The rats were sacrificed 2 h later, and the prostaglandin E_2 contents in right hind feet were measured by prostaglandin E_2 EIA kits. The prostaglandin E_2 content in the gastric mucosa of the control group was 332±24 pg/mg tissue (mean±S.E.M., $n=12$). The prostaglandin E_2 content in the inflamed hind foot of the control group was 16±1.2 ng/paw (mean±S.E.M., $n=16$) after subtracting the prostaglandin E_2 content of the normal group (2.7±0.21 ng/paw, $n=16$).

longer t_{max}, suggesting that the absorption at 5 mg/kg was slower than those at the lower doses.

3.3. In vivo effect on tissue prostaglandin contents

The pharmacokinetic profile showed that CS-706 was well absorbed after oral administration in rats. We then assessed the inhibitory effects of CS-706 on prostaglandin E_2 contents both in stomach (derived from COX-1) and in inflamed tissue (derived from COX-2) and compared with those of indomethacin. This provided a quantitative assessment of the specific inhibition of COX isoforms *in vivo*. The prostaglandin E_2 level in the inflamed tissue was dose-dependently inhibited by CS-706 with an ID_{50} value of 0.073 mg/kg, whereas the prostaglandin E_2 level in the gastric mucosal tissue was inhibited by CS-706 less potently with an ID_{50} value of 1.6 mg/kg. The selectivity ratio ($ID_{50,\ stomach}$/$ID_{50,\ inflamed\ tissue}$) was estimated to be 22 (Table 3). In contrast, no selectivity for either COX isoform was shown by indomethacin in this *in vivo* assay with a selectivity ratio of 1.2.

3.4. In vivo inflammation models

3.4.1. Antinociceptive activity in acute pain model in rats (Randall–Selitto method)

This assay is a rapid and reliable method of measuring the antinociceptive activity of acute inflamed pain, and is frequently used for the evaluation of NSAIDs. In order to investigate the antinociceptive effect of CS-706 in this model, inflammatory pain was introduced in the right hind paws of rats by injecting yeast suspension. The number of antinociceptive positive animals increased at the dose range of 0.0031 to 0.049 mg/kg, and 100% of the animals were antinociceptive positive at doses of 0.049 mg/kg and over. An ED_{50} value was calculated to be 0.0090 mg/kg (95% C.I.: 0.0051–0.017) (Table 4), which was 109 and 27 times more potent than those of celecoxib and rofecoxib at the dose-basis, respectively. CS-706 showed the most potent antinociceptive activity among the COX-2 inhibitors tested (Table 5).

Table 4
Antinociceptive effect of CS-706 on the yeast-induced pain in rats (Randall–Selitto method)

	Dose (mg/kg)	*n*	No. of antinociceptive positive animals	Incidence of positive animals (%)	ED_{50} (mg/kg) (95% C.I.)
CS-706	0.0	17	0	0	
	0.00076	5	0	0	
	0.0031	10	1	10	0.0090
	0.012	10	6	60	(0.0051–
	0.049	10	10	100	0.017)
	0.20	10	10	100	

Male Wistar–Imamichi rats received a single injection of 20% yeast-suspension into the right hind paw. Four and one-half hours later, the animals received an oral administration of CS-706 at the indicated doses or the vehicle. The paws were pressed by a balance pressure device at 0.5, 1 and 2 h after dosing, and pain response was assessed. Antinociceptive positive animals were estimated as described in Materials and methods.

Table 5
ED_{50} values of CS-706 and other compounds in yeast-induced acute pain in rats

	ED_{50} (mg/kg)	95% CI
CS-706	0.0090	0.0051–0.017
Celecoxib	0.98	0.55–1.7
Rofecoxib	0.24	0.13–0.42
Valdecoxib	0.097	0.038–0.25
Etoricoxib	>0.30	

The compounds were administered orally to the rats 4.5 h after yeast-injection to the hind paw. The dose ranges were from 0.0031 to 0.049 mg/kg, 0.20 to 6.3 mg/kg, 0.050 to 3.1 mg/kg, and 0.010 to 1.0 mg/kg for CS-706, celecoxib, rofecoxib, and valdecoxib, respectively. The paws were pressed by a balance pressure device at 0.5, 1 and 2 h after dosing, and pain response was assessed. ED_{50} and the 95% C.I. were calculated based on the incidence of antinociceptive positive animals and the doses. Each experiment was conducted with 5–19 animals.

3.4.2. Antinociceptive activity in chronic pain model in adjuvant-induced arthritis in rats

In order to investigate the antinociceptive effect of CS-706 on chronic pain, adjuvant-induced arthritis was introduced in rats by injecting *M. butyricum* in liquid paraffin emulsion. In this model, chronic pain was well established on Day 18, and the antinociceptive effect was evaluated on that day. The pain scores were decreased time-dependently and dose-dependently after single oral administration of CS-706 at the dose range of 0.10 to 3.2 mg/kg, as shown in Fig. 3. The antinociceptive effect of CS-706 was almost maximal at 2 h after dosing, except for lower doses. In order to assess the earlier effect of the test compounds after dosing, ED_{50} values were estimated from the pain scores at 0.5, 1 and 2 h. CS-706 showed an ED_{50} value of 0.30 mg/kg (95% C.I.: 0.016 – 0.96), which was 25 and 23 times lower than those of celecoxib and rofecoxib, respectively (Table 6).

3.4.3. Prophylactic effect on adjuvant-induced arthritis in rats

This model is frequently used as a chronic arthritis model for measuring the anti-swelling activities of NSAIDs. The prophylactic

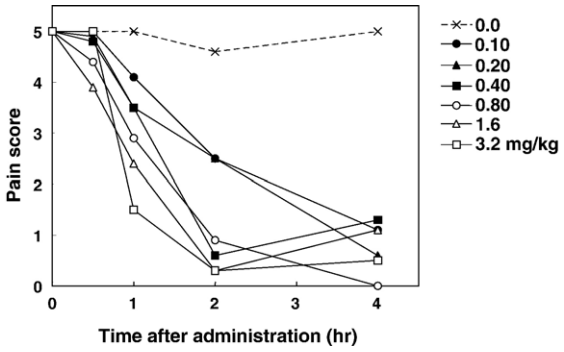

Fig. 3. Antinociceptive effect of CS-706 on adjuvant-induced arthritic pain in Lewis rats. Male Lewis rats received a single, right hind-paw intradermal injection of *M. butyricum* (100 μg/0.05 mL/paw) in liquid paraffin emulsion on Day 0. Animals with discernible pain on paw flexion on Day 18 received single oral doses of CS-706 or the vehicle on the day. Pain response was examined at 0, 0.5, 1, 2 and 4 h after compound administration and pain scores were assessed as described in Materials and methods. Each data point represents the mean of 8 animals.

Table 6
ED_{50} values of CS-706 and other compounds in adjuvant-induced arthritic pain in Lewis rats

	ED_{50} (mg/kg)	95% CI
CS-706	0.30	0.016–0.96
Celecoxib	7.6	4.0–14
Rofecoxib	6.8	3.8–12
Valdecoxib	0.89	0.43–2.4
Etoricoxib	0.56	0.25–1.3

The compounds were administered orally to the adjuvant arthritis rats on Day 18. The dose ranges were from 0.10 to 1.6 mg/kg, 0.80 to 25 mg/kg, 3.1 to 12.5 mg/kg, 0.10 to 3.0 mg/kg, and 0.10 to 3.0 mg/kg for CS-706, celecoxib, rofecoxib, valdecoxib, and etoricoxib, respectively. Pain response was examined at 0.5, 1 and 2 h after dosing and pain scores were assessed. ED_{50} and the 95% C.I. were calculated based on the incidence of antinociceptive positive animals and the doses. Each experiment was conducted with 5–10 animals.

Table 7
ID_{50} values of CS-706 and other compounds in adjuvant-induced arthritis in Lewis rats

	ID_{50} (mg/kg)	95% CI
CS-706	0.10	0.059–0.15
Celecoxib	0.37	0.28–0.49
Rofecoxib	0.21	0.11–0.40
Valdecoxib	0.048	0.031–0.070
Etoricoxib	0.37	0.27–0.55

The compounds were administered orally to the rats once daily from Day 0 to Day 20. The dose ranges were from 0.040 to 5.0 mg/kg, 0.040 to 5.0 mg/kg, 0.040 to 1.0 mg/kg, 0.0080 to 1.0 mg/kg, and 0.040 to 1.0 mg/kg for CS-706, celecoxib, rofecoxib, valdecoxib, and etoricoxib, respectively. The volume of the adjuvant-injected foot was measured on Day 21. ID_{50} and the 95% C.I. were calculated based on the % inhibition and the doses. Each experiment was conducted with 5–12 animals.

effect of CS-706 was investigated using this rat chronic inflammation model. As shown in Fig. 4, the feet of the vehicle-treated rats swelled dramatically starting at about 10 days after the adjuvant injection, and the volume reached the maximum around Day 18. The foot swelling was dose-dependently inhibited by CS-706 from the dose of 0.040 mg/kg/day, and the inhibitory effect reached a plateau at doses over 1.0 mg/kg/day. ID_{50} values of CS-706 and other compounds were calculated from the swelling on Day 21 (Table 7). The ID_{50} of CS-706 was 0.10 mg/kg (95% C.I.: 0059–0.15). CS-706 was one of the potent inhibitors among the COX-2 inhibitors tested, together with valdecoxib, in this chronic inflammation model.

3.4.4. Therapeutic effect on adjuvant-induced arthritis in rats

The therapeutic effect of CS-706 was evaluated in well-established adjuvant-induced arthritis rats. CS-706 was administered orally to the rats twice a day for 7 days from Day 18 to Day 24 (the maximum swelling of the feet was observed on Days 18–24). Fig. 5 indicates the time course of the volume of the swelled feet. CS-706 reduced the foot swelling dose-dependently and time-dependently. On Day 25, the swelling volume was reduced by 22.3%, 41.8%, 54.7% and 53.5% at doses of 0.040, 0.20, 1.0 and 5.0 mg/kg/day, respectively. The inhibitory effect of CS-706 reached a plateau at doses over 1.0 mg/kg/day.

3.5. Gastric Lesions

Gastric mucosal lesion formation was investigated in rats with a single oral dose of CS-706. The mean length of the hemorrhage lesions in the gastric mucosa was 0.70±0.40 mm (mean±S.E.M.), and the incidence of positive animals was 20% (1/5) at 100 mg/kg CS-706 (n=5). UD_{50} value of CS-706 was more than 100 mg/kg (Table 8). In contrast, oral administration of the non-selective inhibitors indomethacin and Diclofenac–Na caused dose-dependent lesion formation at dose ranges of 3.0- 15 mg/kg and 4.4-22.5 mg/kg, respectively. The length of the lesions at a dose of 10 mg/kg reached 8.4±1.4 mm and 5.3±2.1 mm for indomethacin and Diclofenac–Na, respectively. UD_{50} values of indomethacin

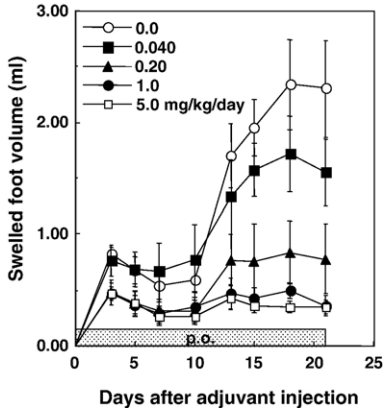

Fig. 4. Prophylactic effect of CS-706 on the development of adjuvant arthritis in Lewis rats. Female Lewis rats received a single, right hind-paw intradermal injection of *M. butyricum* (100 mg/0.05 ml/paw) in a liquid paraffin emulsion on Day 0. CS-706 was orally administered at 0.040, 0.20, 1.0 and 5.0 mg/kg/day once a day from Day 0 to Day 20 after the adjuvant-injection. The vehicle was administered to the control group (0 mg/kg/day). The swelled foot volume of the adjuvant-injected foot was determined on Days 3, 5, 7, 10, 13, 15, 18 and 21 by a plethysmometer. Each data point represents the mean±S.D. of 5 animals.

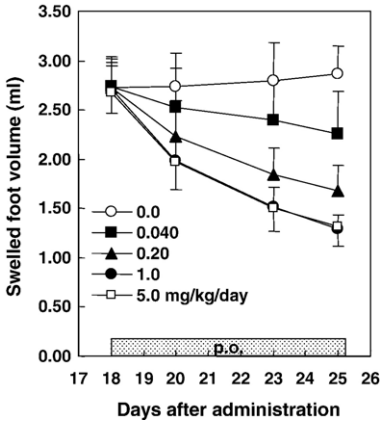

Fig. 5. Therapeutic effect of CS-706 on the established adjuvant arthritis in Lewis rats. Female Lewis rats received a single, right hind-paw intradermal injection of *M. butyricum* (100 μg/0.05 ml/paw) in a liquid paraffin emulsion on Day 0. CS-706 was orally administered at 0.040, 0.20, 1.0 and 5.0 mg/kg/day twice a day from Day 18 to Day 24. The vehicle was administered to the control group (0 mg/kg/day). The swelled foot volume of the adjuvant-injected foot was determined on Days 18, 20, 23 and 25. Each data point represents the mean±S.D. of 8 animals.

Table 8
UD_{50} values of CS-706 and other compounds in gastric mucosal lesion formation in rats

	Dose (mg/kg)	n	Total length of hemorrhage lesions (mm, mean ± S.E.M.)	Incidence of lesion positive animals (%)	UD_{50} (mg/kg) (95% C.I.)
CS-706	0.0	5	0.0±0.0	0	
	100	5	0.70±0.40	20	>100
Indomethacin	0.0	5	0.0±0.0	0	
	3.0	5	0.40±0.19	0	
	4.4	5	1.3±0.60	20	5.4
	6.7	5	6.4±2.5	80	(3.2–7.8)
	10	5	8.4±1.4	100	
	15	5	27±5.0	100	
Diclofenac–Na	0.0	5	0.0±0.0	0	
	4.4	5	0.50±0.39	20	
	6.7	5	3.1±1.5	60	6.3
	10	5	5.3±2.1	80	(3.2–9.0)
	15	5	11±3.4	100	
	22.5	5	26±4.4	100	

A single oral dose of the test compounds or the vehicle was administered to male Wistar–Imamichi rats after overnight fasting. The rats were sacrificed 3.5 h after administration and the stomachs were removed. The mucosa was then examined for the presence of hemorrhage lesions as described in Materials and methods.

and Diclofenac–Na were calculated to be 5.4 mg/kg and 6.3 mg/kg, respectively (Table 8).

4. Discussion

There have been substantial attempts to identify COX-2-selective inhibitors with anti-inflammatory properties without the adverse effects of traditional NSAIDs since the discovery of two isoforms of COX (COX-1 and COX-2). This study describes the preclinical pharmacology of a novel COX-2-selective inhibitor, CS-706, a sulfonamide-containing diphenylpyrrole derivative, with potent antinociceptive and anti-inflammatory activity. CS-706 is 7.1-fold more selective for COX-2 than for COX-1 in a whole blood assay. Consistent with its weak activity on COX-1, the gastric tolerability of CS-706 is significantly better than those of indomethacin and Diclofenac–Na which nonselectively inhibit COX-1 and COX-2.

The human whole blood COX assay conducted here is based on the production of prostaglandins induced by LPS (COX-2 dependent) and Ca^{2+} ionophore (COX-1 dependent) in whole blood. It is often recognized as the appropriate assay to provide an index of COX-1/-2 selectivity *in vitro*, since inhibition of cyclooxygenase isozymes is measured in physiological conditions taking into account the binding of the drugs to plasma proteins and un-targeted cells. This procedure has often been used in measuring COX-2 selectivity of the compounds *in vitro* and applied to clinical studies of COX-2 selective inhibitors as an *ex vivo* assay. In our assay, CS-706 demonstrated higher COX-2 selectivity (7.1-fold) than those of conventional NSAIDs, but lower or more moderate selectivity among COX-2 inhibitors similar to celecoxib (8.9-fold). This low or moderate selectivity of CS-706 seems to be sufficient to spare gastrointestinal toxicity, since celecoxib itself has been shown to have low ulcerogenic activity in clinical use (Singh et al., 2006).

Similar to the result in the human whole blood assay, potent and selective inhibition towards COX-2 enzyme by CS-706 was confirmed in a human recombinant enzyme assay. However, the recent COX-1/2 inhibitors are reported to exhibit reversible and time-dependent inhibition of cyclooxygenase enzymes (Esser et al., 2005). Therefore, further studies will be required to elucidate the mechanism of CS-706 actions in terms of competitiveness, reversibility, and time-dependency for inhibition of cyclooxygenase enzymes.

The pharmacokinetic profile of CS-706 was then examined prior to the *in vivo* pharmacological studies in rats. CS-706 was rapidly absorbed and its plasma concentration reached maximum between 1–2 h after oral administration. It showed dose-proportional exposure with $t_{1/2}$ of 3–6 h in rats. This encouraged further animal studies, and we directly assessed the *in vivo* COX-2 selectivity of CS-706 in terms of the inhibition of tissue prostaglandin contents derived from either COX-1 (gastric mucosa) or COX-2 (inflamed hind paw) and compared it with that of indomethacin. The *in vivo* selectivity of CS-706 was 22-fold and more selective than indomethacin (1.2-fold). It was demonstrated in human clinical studies that CS-706 potency (EC_{50}) derived from *ex vivo* whole blood assays was 397 ng/ml (=1.1 μM) for COX-1 and 20 ng/ml (=0.056 μM) for COX-2 (Rohatagi et al., 2007). This selectivity (397/20=20-fold) was quite consistent with the *in vivo* tissue selective inhibition in rats (22-fold) described in this article. Taken together, these results show that CS-706 is well absorbed after oral administration and selective for COX-2 both *in vitro* and *in vivo*.

CS-706 was therefore investigated in typical inflammatory animal models used in studying NSAIDs, and it was found to be one of the most potent COX-2 inhibitors among the comparators. First, CS-706 showed the most potent antinociceptive activity with an ED_{50} value of 0.0090 mg/kg in a yeast-induced inflammatory acute pain model (Randall–Selitto method), which has frequently been used in antinociceptive assays of NSAIDs. CS-706 also showed a potent antinociceptive activity with an ED_{50} value of 0.30 mg/kg in the established chronic pain model of adjuvant-induced arthritis in rats. Second, CS-706 inhibited foot swelling prophylactically with an ID_{50} value of 0.10 mg/kg/day, and decreased the foot swelling in established arthritis therapeutically in a dose range of 0.040 to 1.0 mg/kg/day in adjuvant-induced arthritis in rats. On the other hand, with regard to ulcerogenic activity, which is one of the major adverse events of NSAIDs, CS-706 showed little activity even at a dose of 100 mg/kg. In the same assay, indomethacin and Diclofenac–Na showed UD_{50} values of 5.4 and 6.3 mg/kg, respectively. Thus, CS-706 is a potent antinociceptive and anti-inflammatory compound with minimal gastric toxicity.

In a yeast-induced inflammatory pain model (Randall–Selitto method), CS-706 showed an ED_{50} value of 0.0090 mg/kg, which was significantly lower compared to the ED_{50} of the adjuvant arthritis pain model (0.30 mg/kg) or the ID_{50} s of adjuvant arthritis inflammation models. The exact reason is not known, however, the yeast-induced pain model is an acute model and may be different from the adjuvant arthritis chronic model perhaps because the drugs

are able to distribute to the inflamed sites more easily and/or the threshold of drug concentration to show antinociceptive effect is lower in this acute model. Consistent with this explanation, most other COX-2-selective inhibitors (e.g. celecoxib, rofecoxib and valdecoxib) examined in this study also required lower doses to show efficacy in this model compared to the arthritis pain model. Furthermore, in the acute assay system, antinociceptive effect of the drugs was assayed soon after administration within 2 h. It was revealed from the pharmacokinetic assay that CS-706 is rapidly absorbed and reaches the t_{max} approximately 1 h after administration. Therefore, CS-706 may be sensitive in this assay because the assay is more adequate for drugs with rapid onset. One piece of evidence to support this sensitivity of CS-706 is the result of the *in vivo* inhibition of prostaglandin in inflamed tissue in the acute pain model: a single dose of CS-706 inhibited the prostaglandin E_2 content in the inflamed tissue by 45% at a low dose of 0.030 mg/kg, although this dose is still 3.4 times higher than the ED_{50} of 0.0090 mg/kg. In any case, further studies will be required to elucidate this issue more precisely.

It has been reported that COX-2-selective inhibitors are associated with improved gastrointestinal tolerability compared with nonselective NSAIDs (Masferrer et al., 1994; Seibert et al., 1994). In the present study, we used visual observation to evaluate gastric lesions in rats given a single dose of CS-706. This is a simple and quick method for determining acute gastric mucosal injury and has been frequently used in gastric tolerability studies of NSAIDs (Hitchens et al., 1967; Shriver et al., 1975). CS-706 showed little activity even at a dose of 100 mg/kg which is 100-fold higher than the maximum effective dose in the therapeutic experiment in adjuvant arthritis (1.0 mg/kg). Another commonly used assay in detecting gastrointestinal integrity is ^{51}Cr-excretion assay after systemic administration of either ^{51}Cr-EDTA or $^{51}CrCl_3$. However, we did not determine the effect of CS-706 on the intestine in the study. Also, repeated dose experiments have not been conducted for CS-706. Therefore, the results obtained here are limited for predicting ulcerogenic effect of this agent. However, a gastrointestinal tolerability study of CS-706 has been conducted in humans, in which CS-706 or naproxen was administered for 7 days and tolerability was assessed by upper gastrointestinal endoscopy (Moberly et al., 2007a). CS-706 showed a gastric safety profile similar to that of placebo and significantly superior to that of naproxen. Accordingly, CS-706 seems to possess a potential for superior gastrointestinal safety compared to traditional NSAIDs.

Recent controlled clinical studies have indicated that use of COX-2-selecitive inhibitors is associated with an increased risk of cardiovascular events (Bresalier et al., 2005; Nussmeier et al., 2005), which has led to the withdrawal of several COX-2-selective inhibitors from the market. However, the risk of cardiovascular events may vary within individual COX-2-selective inhibitors; recent meta-analyses of randomized and nonrandomized trials suggest a greater risk for cardiovascular events with rofecoxib than with celecoxib (Graham et al., 2005; Brophy, 2005; Jones, 2005; McGettigan and Henry, 2006; Solomon et al., 2006). Recently, nonclinical distribution studies have indicated new evidence regarding the cardiovascular risk of rofecoxib, demonstrating that a considerable amount of radiolabelled rofecoxib, but not CS-706 or celecoxib, was retained by and accumulated in the thoracic aorta, the aortic arch and the coronary artery as a consequence of covalent binding to elastin in rats after oral administration (Oitate et al., 2006). Furthermore, rofecoxib was found to cause degradation of the elastic fiber system, which may lead to an increased risk of cardiovascular events after long term treatment with rofecoxib (Oitate et al., 2007). This finding may offer a possible mechanism for differences in cardiovascular risk among COX-2-selective inhibitors. Given the therapeutic importance of COX-2-selective inhibitors, further investigation of agents such as CS-706 may be warranted.

CS-706 has been investigated in phase I studies and a phase IIa study (Rohatagi et al., 2007; Moberly et al., 2007a,b). Early clinical results indicate that CS-706 is well tolerated in healthy subjects. Consistent with the preclinical pharmacological activities, CS-706 showed significantly greater analgesic efficacy compared with celecoxib in a phase IIa study in healthy subjects experiencing moderate or severe dental pain, following the surgical extraction of teeth (Moberly et al., 2007b). As mentioned above, CS-706 also showed a gastric safety profile similar to that of placebo and significantly superior to that of naproxen in a 7-day repeated dose study (Moberly et al., 2007a).

In conclusion, CS-706 is a COX-2-selective inhibitor with modest but significant COX-2 selectivity, and has potent anti-inflammatory and antinociceptive activities and a favorable gastrointestinal safety profile. CS-706 is now in phase II clinical trials for treatment of inflammation and cancers as a prospective COX-2-selective inhibitor.

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