

Covalent binding of radioactivity from [¹⁴C]rofecoxib, but not [¹⁴C]celecoxib or [¹⁴C]CS-706, to the arterial elastin of rats

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Abbreviations:

COX-2, cyclooxygenase-2; CV, cardiovascular; NSAID, non-steroidal anti-inflammatory drug;

APPROVe, Adenomatous Polyp Prevention on VIOXX; PGI₂, prostacyclin; Tx, thromboxane; TCA,

trichloroacetic acid; K_p, the ratio of concentration in tissue to that in plasma (tissue/plasma).

Abstract

Rofecoxib is a cyclooxygenase-2 (COX-2) inhibitor which has been withdrawn from the market due to an increased risk of cardiovascular (CV) events. With a special focus on the arteries, the distribution profiles of radioactivity in rats orally administered [^{14}C]rofecoxib were investigated in comparison with two other COX-2 inhibitors, [^{14}C]celecoxib and [^{14}C]CS-706, a novel selective COX-2 inhibitor. Whole-body autoradioluminography and quantitative determination of the tissue concentrations showed that considerable radioactivity is retained by and accumulated in the thoracic aorta of rats after oral administration of [^{14}C]rofecoxib, but not [^{14}C]celecoxib or [^{14}C]CS-706. Acid-, organic solvent- and proteolytic enzyme-treatments of aorta retaining high levels of radioactivity from [^{14}C]rofecoxib demonstrated that most of the radioactivity is covalently bound to elastin. In agreement with this result, the radioactivity was found to be highly localized on the elastic fibers in the aorta by microautoradiography. The retention of radioactivity on the elastic fibers was also observed in the aortic arch and the coronary artery. These findings indicate that [^{14}C]rofecoxib and/or its metabolite(s) are covalently bound to elastin in the arteries. These data are consistent with the suggestion of modified arterial elasticity leading to an increased risk of CV events after long-term treatment with rofecoxib.

Introduction

Rofecoxib (VIOXX) is a potent and highly selective cyclooxygenase-2 (COX-2) inhibitor which has been widely used as a non-steroidal anti-inflammatory drug (NSAID). However, this drug was withdrawn from the worldwide market due to an increased risk of cardiovascular (CV) events found in the Adenomatous Polyp Prevention on VIOXX (APPROVe) trial, which was conducted for the prevention of the recurrence of colorectal polyps and included 2,600 patients with no history of CV disease before enrollment (Merck, 2004). The study had originally been intended to last for 3 years, but was halted in mid-course due to the increased CV risks among patients in the group that was taking 25 mg rofecoxib, in which the incidence of risk was more than twice that in the group taking the placebo.

Recently, it has been reported that other selective COX-2 inhibitors (*e.g.* etoricoxib, parecoxib/valdecoxib and celecoxib) and non-selective NSAIDs (*e.g.* naproxen) may also have a potential for increased CV risk (Aldington, 2005; Ott, 2003; Nussmeier, 2005; Krotz, 2005; FDA Advisory Committee.com, 2004; FDA, 2004; Bombardier, 2000; NIH, 2004). However, rofecoxib differs in the following manner: 1) a significantly greater frequency and higher odds of CV events (Mamdani, 2004; Graham, 2005; Kimmel, 2005; Solomon, 2004a); 2) a shorter period and a lower dose (even at the clinical dose) leading to the incidence of CV events; 3) an earlier-onset and a greater hypertensive effect correlating closely with CV risk (Solomon, 2004b; Wolfe, 2004; Brinker,

2004; Fredy, 2005). Therefore, it is suggested that rofecoxib could have distinctive mechanisms or more potent toxic activity leading to CV risks, in comparison with other selective COX-2 inhibitors or non-selective NSAIDs. Consequently, it is vital to investigate rofecoxib by comparing it with other selective COX-2 inhibitors in order to clarify its relevance to increased CV events.

Several hypotheses have been proposed to explain the mechanism for the increased risk of CV events with rofecoxib. McAdam (1999) suggested that the endothelial prostacyclin (PGI₂)/thromboxane (Tx) A₂ imbalance theory; whereby the CV complications caused by selective COX-2 inhibitors might be partially due to an imbalance of the concentration ratio of two prostanoids with major CV actions - PGI₂, a vasodilator and inhibitor of platelet aggregation, and TxA₂, a vasoconstrictor and promotor of platelet aggregation. That is, selective COX-2 inhibitors diminish the production of PGI₂ in the endothelium, but not TxA₂ in the platelets, so that the relative concentration of TxA₂ increases around the affected area, which might increase the risks. Walter (2004) proposed the pro-oxidant effect theory; whereby rofecoxib, which is a sulfone-type COX-2 inhibitor, but not celecoxib (sulfonamide-type COX-2 inhibitor) or nonselective NSAIDs, promotes oxidative damage to LDL and phospholipids *in vitro*, and this action might lead to atherogenesis *in vivo*. In addition, Pope (1993) and Johnson (1994) proposed the blood pressure elevation theory; in which NSAIDs and selective COX-2 inhibitors disrupt the production of prostaglandins, which play an important homeostatic role in the kidney. The resulting sodium and water retention can

contribute to blood pressure elevation. The higher risk potential of rofecoxib on the CV events can be supported only by Walter's hypothesis. However, it may be not enough to distinguish rofecoxib from other COX-2 inhibitors and NSAIDs in terms of the risks.

CS-706 is a novel sulfonamide-type selective COX-2 inhibitor, having the strongest anti-inflammatory activity in animal models in its class (Sankyo Co., Ltd., data on file, manuscript in preparation). This drug is currently in phase 2 studies in the USA and its efficacy and safety have been demonstrated (Sankyo Co., Ltd., data on file, manuscript in preparation).

In the present study, we investigated the distribution profiles of radioactivity after oral administration of [^{14}C]rofecoxib to rats, especially in its retention by and accumulation in the thoracic aorta, by comparing it with [^{14}C]celecoxib and [^{14}C]CS-706 as references (Figure 1). Aorta samples were collected after administration of [^{14}C]rofecoxib, and treated with acid, organic solvent and proteolytic enzymes to demonstrate that [^{14}C]rofecoxib and/or its metabolite(s) are covalently bound to a protein of the aorta. Microautoradiographic observation of the radioactivity localized in the aorta was also conducted.

Materials and Methods

Chemicals and Reagents

[¹⁴C]Rofecoxib (59 mCi/mmol for microautoradiography and 17 mCi/mmol for the other studies), [¹⁴C]celecoxib (13 mCi/mmol) and [¹⁴C]CS-706 (14 mCi/mmol) were synthesized at Amersham Biosciences Corp. (Piscataway, NJ, USA, Figure 1). The radiochemical purity of these compounds was more than 98% by radiodetection-HPLC analysis. Non-labeled rofecoxib, celecoxib and CS-706 were synthesized at Sankyo Co., Ltd. (Tokyo, Japan). All other reagents and solvents used were commercially available and were of extra pure, guaranteed or HPLC grade.

Dosing of animals

Male Sprague-Dawley rats (7 weeks old, Charles River Japan, Inc., Yokohama, Japan) were used after one or more weeks of acclimatization. Rats were housed in a temperature-controlled room with a 12-h light/dark cycle. Their body weights ranged from 160 g to 260 g at the start of administration. For the single oral administration, rats were fasted overnight before dosing and through 8 h post-dose. For the repeated administrations, rats were fasted only on the first day. Water was available *ad libitum* throughout the study.

[¹⁴C]Rofecoxib was orally administered at 2 mL/kg as a solution in polyethylene glycol 400.

[¹⁴C]Celecoxib and [¹⁴C]CS-706 were orally administered at 2 mL/kg as a solution in a mixture of N, N-dimethylacetamide, Tween 80 and distilled water (5/20/75, v/v/v). The dosing solutions were prepared shortly before dosing at the same specific radioactivity within the same study (ranged 68 - 370 μCi/kg body weight).

Whole-body autoradioluminography

Rats (one animal at each time point) were euthanized by deep anesthesia with diethyl ether at 0.5, 2, 6, 24 and 48 h after single oral administration (2 mg/kg) of [¹⁴C]rofecoxib, [¹⁴C]celecoxib or [¹⁴C]CS-706, at 48 h after 3-day repeated administrations of [¹⁴C]rofecoxib (2 mg/kg) and at 48 h and 10 days after 7-day repeated administrations of [¹⁴C]rofecoxib (2 mg/kg). The rats were frozen in n-hexane/dry ice, embedded in a gel of carboxymethyl cellulose (*ca.* 5%, w/v), and frozen again in n-hexane/dry ice. The frozen blocks were sliced with Cryomacrocut (CM3600, Leica Microsystems Nussloch GmbH, Nussloch, Germany) at around -25°C to prepare whole-body sections of 50-μm thickness. The whole-body sections obtained were freeze-dried at around -25°C for 48 h. After freeze-drying, the sections were covered with a protective film (4 μm, DIAFOIL membrane, Mitsubishi Polyester Film Corp., Tokyo, Japan) and placed in contact with Imaging Plates (BAS-MS2040, Fuji Photo Film Co., Ltd., Tokyo, Japan) for about 24 h. Finally, the Imaging Plates were subjected to image analysis using a Bio Imaging Analyzer (BAS-2500, Fuji

Photo Film Co., Ltd.) to obtain whole-body autoradioluminograms (resolution: 50 μ m, gradation: 256, sensitivity: 10,000, latitude: 4).

Quantitative tissue distribution

After single oral administration of [14 C]rofecoxib, [14 C]celecoxib or [14 C]CS-706 to the rats (2 mg/kg, N=3), blood samples were taken from the abdominal aorta under diethyl ether anesthesia at 0.5, 2, 6, 24 and 48 h post-dose. Subsequently, the liver, kidney, lung, aorta, cartilage (auricular) and skin (back) were collected from each carcass. After repeated administrations of [14 C]rofecoxib to rats (2 mg/kg, N=3) for 3 days or 7 days, the blood and above described tissues were collected at 48 h post-dose. Blood samples were centrifuged (4°C, 1,680 g, 10 min) to obtain plasma samples.

Acid-, organic solvent- and proteolytic enzyme-treatments of thoracic aortas

At 24 h after single oral administration of [14 C]rofecoxib (2 mg/kg, N=10), rats were euthanized by exsanguination under diethyl ether anesthesia and the aorta samples were collected, pooled and weighed. The samples were homogenized in isotonic saline (5% w/v) using a motor-driven homogenizer, and 0.2 mL of the homogenate was removed for determination of the radioactivity. To the remaining homogenate, 30 mL of 0.9 M trichloroacetic acid (TCA) was added to precipitate the proteins. Then, the mixture was centrifuged at 2,000 g at room temperature for 10 min. The

supernatant was discarded, and the precipitate was washed by resuspension and centrifugation successively with 0.6 M TCA, 80% (v/v) methanol and 100% methanol. This process was repeated twice.

The resulting precipitate (wet weight: 636.9 mg) was resuspended in 5 mL of 0.1 M Tris-HCl buffer (pH 8.0) containing collagenase (5,000 units/mL; from *Clostridium histolyticum*, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and incubated at 37°C for 16 h. After centrifugation (2,000 g, room temperature, 10 min), the supernatant containing collagen digests was collected. The precipitate was suspended in 3 mL of methanol, mixed on a vortex shaker, and recentrifuged. The supernatants were collected as methanol-washings. This process of washing was repeated three times. The residue was air-dried, resuspended and incubated in 5 mL of 0.1 M Tris-HCl buffer (pH 8.5) containing elastase (600 units/mL; from porcine pancreas, Wako Pure Chemical Industries, Ltd.) at 37°C for 16 h. After the same washing process as described for the collagenase-treatment, the remaining dried residue was resuspended and incubated in 6 mL of 0.1 M sodium phosphate buffer (pH 7.5) containing pronase (1,000 units/mL; from *Streptomyces griseus*, Merck, Darmstadt, Germany) at 37°C for 16 h. In the same manner as the collagenase- and elastase-treatments, the precipitate after pronase-treatment was washed with methanol. The combined digests and methanol-washings collected after each enzyme-treatment were dried *in vacuo*.

Microautoradiography

A rat was euthanized by deep anesthesia with diethyl ether at 48 h after 7-day repeated oral administrations of [¹⁴C]rofecoxib (2 mg/kg), and the thoracic aorta, aortic arch and coronary artery were collected. These arterial tissues were fixed in Bouin's fixative fluid and then embedded in paraffin wax. Paraffin sections of 4- μ m thickness were cut with a microtome (ML-357, Yamato Kohki Industrial Co., Ltd., Saitama, Japan), and mounted on a glass slide. The slides on the sections were coated with nuclear emulsion (Kodak NTB-3, Eastman Kodak Co., Rochester, NY, USA) under safe lighting. After exposure for one month, the slides were developed and then stained with hematoxylin and eosin (HE) and Weigert's elastica and Masson's trichrome according to the common procedures.

Radioactivity measurements

An aliquot of the plasma sample was placed in a vial and mixed with a tissue solubilizer NCS-II (Amersham International plc, Little Chalfont, Buckinghamshire, UK, 1 mL). Each proteolytic fraction of the aorta was solubilized each in 1 mL of NCS-II, and an aliquot of each solution was transferred to a vial. Tissues or organs were measured for the wet weight, and solubilized each in 2 mL of NCS-II under constant shaking at *ca.* 55°C. In the case of colored tissues, decolorization

with 30% hydrogen peroxide (0.3 mL) was done to avoid color quenching in the liquid scintillation counting. After decolorization and solubilization, these samples were mixed with 10 mL of a liquid scintillator HIONIC-FLUOR (PerkinElmer Life and Analytical Sciences, Boston, USA) and were subjected to radioactivity measurement by a liquid scintillation counter Model 2300TR (PerkinElmer Life and Analytical Sciences).

The radioactivity in the plasma and tissues was calculated as an equivalent value of [^{14}C]labeled compound, and expressed as a concentration per mL (for plasma) or per g (for tissues). The ratio of the concentration in the tissue to that in the plasma was calculated as a K_p value. The radioactivity value of each proteolytic fraction of the aorta was converted to a percentage of the total tissue-bound radioactivity, which is not extractable by TCA- and methanol-treatments.

Statistical analysis

Statistical analysis of the experimental data was performed using a Student's t -test. $P < 0.05$ was regarded as statistically significant.

Results

Whole-body autoradioluminography

After single oral administration of [^{14}C]rofecoxib, [^{14}C]celecoxib or [^{14}C]CS-706, high levels of radioactivity were observed throughout the whole body, and there was no marked difference in the distribution profile among the three compounds up to 6 h, as representatively shown in the autoradioluminograms at 2 h (left panels, Figure 2). At 24 and 48 h after the administration of [^{14}C]celecoxib or [^{14}C]CS-706, the radioactivity was only observed in the gastrointestinal contents. On the other hand, in the case of [^{14}C]rofecoxib, high levels of radioactivity were observed in the thoracic aorta and liver, as representatively shown in the autoradioluminograms at 48 h (right panels, Figure 2). Furthermore, at 48 h after 3- or 7-day repeated administrations of [^{14}C]rofecoxib, the radioactivity appeared to have accumulated in the aorta and interspinal ligaments in a dosing frequency-dependent manner (Figure 3(A and B)). In addition, the radioactivity in these tissues was still clearly observed 10 days after the 7-day repeated administrations (Figure 3C), when the radioactivity was almost completely cleared from the body.

Quantitative tissue distribution

The concentrations of radioactivity in the plasma and tissues after oral administration of

[¹⁴C]rofecoxib, [¹⁴C]celecoxib or [¹⁴C]CS-706 to rats are shown schematically in Figure 4. After single administration of [¹⁴C]rofecoxib (Figure 4A), radioactivity was observed in all the tissues examined at 0.5 h, reached the maximum concentrations (C_{max}) at 0.5 or 2 h, and thereafter the levels in the plasma and tissues except the aorta gradually declined in parallel. The C_{max} of the radioactivity in the plasma was 1.1 μg equivalents/mL at 0.5 h post-dose, while the concentrations in the thoracic aorta, cartilage (auricular) and liver were 0.8, 0.7 and 4.4 μg equivalents/g with K_p values of 0.8, 0.7 and 4.8, respectively. At 48 h post-dose, a considerably high concentration of radioactivity was observed in the aorta (0.3 μg equivalents/g), with a K_p value of 19.1 and was almost the same level as that observed at 6 and 24 h post-dose (0.3 and 0.5 μg equivalents/g, respectively). The cartilage retained a relatively high concentration at 48 h, with a K_p value of 3.9, which was about 6 times higher than that at 0.5 h post-dose. On the other hand, the K_p values of the liver were not so different between 0.5 h and 48 h post-dose. As shown in Figure 4B, after 3- or 7-day repeated administrations (once daily) of [¹⁴C]rofecoxib, the levels of radioactivity in the aorta at 48 h post-dose were increased depending on the dosing frequency. The concentrations in the aorta at 48 h after 1-, 3-, and 7- day repeated administrations were 0.3, 0.7 and 1.8 μg equivalents/g with K_p values of 19.1, 33.9 and 52.7, respectively.

After single oral administration of [¹⁴C]celecoxib or [¹⁴C]CS-706 (Figure 4(C or D)), the radioactivity was distributed to all the tissues examined at 0.5 h post-dose and the time to reach the

C_{\max} (T_{\max}) was between 0.5 and 2 h, with a plasma concentration of 0.5 or 0.4 μg equivalents/mL at 2 h post-dose. Thereafter, the plasma concentration declined almost in parallel with the decrease in the concentrations in all the tissues, including the aorta. At 48 h, negligible concentrations were observed in all the tissues.

Acid-, organic solvent- and proteolytic enzyme-treatments of thoracic aortas

The combined thoracic aorta samples collected at 24 h after single oral administration of [^{14}C]rofecoxib to the rats were treated by successive extraction with 0.6 M TCA, 80% (v/v) methanol and 100% methanol, and 71.5% of the radioactivity that existed initially in the aorta remained in the final precipitate fraction, which was considered to be bound covalently to proteins in the aorta. The resultant precipitated fraction was enzymatically treated successively with collagenase, elastase and pronase. As shown in Table 1, most of the radioactivity (91.4% of total) was recovered in the elastolytic fraction. The radioactivity recovered in the fractions after collagenase- and pronase-treatments was 4.4% and 2.7%, respectively, and the radioactivity in the residue after the treatments with the proteolytic enzymes was 1.4%.

Microautoradiography

The intra-arterial localization of the radioactivity at 48 h after 7-day repeated oral administrations

of [^{14}C]rofecoxib to rats was investigated by microautoradiographs with elastin staining, as shown in Figure 5. In the thoracic aorta and aortic arch (Figure 5(A and B)), the three basic layers (intima, media and adventia) were seen, and the elastic fibers made visible by elastin-staining mainly existed in the media. The number of elastic fibers in the aortic arch was more than that found in the thoracic aorta. The radioactivity detected as black silver grains was mainly located on the elastic fibers (media), not on the intima or adventia, in good agreement with the results of the enzymatic fractionation (Table 1). In the coronary artery (Figure 5C), radioactivity was also detected on the elastic fibers even though the elastic fiber layer was much thinner and fainter than those of the thoracic aorta and aortic arch.

Discussion

Whole-body autoradioluminography and quantitative determination of the tissue concentration by dissection method after single oral administration of [^{14}C]rofecoxib, [^{14}C]celecoxib or [^{14}C]CS-706 showed that a high level of radioactivity was retained up to 48 h in the thoracic aorta after administration of [^{14}C]rofecoxib, while the radioactivity in other tissues and plasma including the aorta was eliminated in parallel after administration of [^{14}C]celecoxib and [^{14}C]CS-706 (Figures 2, 4(A, C and D)). Repeated administrations of [^{14}C]rofecoxib for 3 or 7 days resulted in an accumulation of radioactivity in the thoracic aorta in a dosing frequency-dependent manner (Figures 3(A and B) and 4B). Similar retention and accumulation of radioactivity were also observed in the interspinal ligament and auricular cartilage. These results suggested that [^{14}C]rofecoxib and/or its metabolite(s), but not [^{14}C]celecoxib or [^{14}C]CS-706, are bound covalently to proteins in these tissues, especially in the aorta, with a high-binding capacity.

Rofecoxib has a 5-member lactone ring (3-phenyl-2(5*H*)-furanone) in its structure, whereas celecoxib and CS-706 have no such lactone ring (Figure 1). Recently, Reddy and Corey (2005) proposed the following mechanism for chronic human toxicity such as CV events. That is, the lactone ring in rofecoxib is capable of undergoing spontaneous oxidation as it circulates to the vasculature and oxygenated tissues *in vivo*, and one of the resulting metabolites, its maleic anhydride form, has the potential to react with nucleophilic groups in biomolecules, especially amino acids.

Furthermore, it is also thought to be possible that the lactone in it can be directly bound to amino acid side chains of biomolecules by enzyme-catalyzed trans-esterification. However, if these hypotheses are correct, it is difficult to explain why the radioactivity is retained only in a limited number of tissues, such as the aorta, ligament and cartilage.

Regarding the tissues in which the radioactivity was retained and accumulated (aorta, ligament and auricular cartilage), it was noted that they are all representative connective tissues that have abundant elastin and collagen in their extracellular matrices. Assuming that rofecoxib and/or its metabolite(s) bind covalently to elastin or collagen, its specific distribution to connective tissues would be well explained. Based on this hypothesis, an enzymatic fractionation of the aorta was conducted with elastase and collagenase. At 24 h after single oral administration of [^{14}C]rofecoxib to rats, most of the aortic radioactivity (71.5% of total) came from the covalently bound fraction which was not extracted by the TCA- and methanol-treatments. As shown in Table 1, almost all of the radioactivity bound to the aorta was recovered in the elastolytic fraction. In addition, a microautographic study also demonstrated that the majority of the radioactivity was clearly located on an elastin-rich region in the thoracic aorta and aortic arch (Figure 5(A and B)), in good agreement with the results of the enzymatic fractionation. Radioactivity was also detected on the elastic fibers of the coronary artery (Figure 5C), which had much thinner and fainter elastic fibers than the thoracic aorta and aortic arch. These findings strongly indicate that [^{14}C]rofecoxib and/or its

metabolite(s) are covalently bound to the elastin in the arteries.

Elastin is a key extracellular matrix protein that is critical to the elasticity and resilience of the connective tissues, and constitutes around 50% of the aorta (dry mass), 66% of the ligaments and 4 - 30% of the cartilage (ear) (Starcher and Galione, 1976; Keith, 1977). As well, the pericardium and the heart valves consist of elastin (Elbahnasy, 1998; Vesely, 1998). Its structural and functional destruction is clinically relevant to CV diseases such as hypertension (Martyn and Greenwald, 1997; D'Armiento, 2003), aneurysm (Inci and Spetzler, 2000), atherosclerosis (Sandberg, 1981), Marfan syndrome (Abraham, 1982), pseudoxanthoma elasticum (PXE) (Raybould, 1994), supraaortic stenosis (SVAS) (Li, 1997) and Williams syndrome (Lowery, 1995). In addition, elastin has a very slow turnover rate with an estimated half-life of about 70 years (Petersen, 2002). It is impossible to completely recover elastin function once it has been damaged (Rucker and Dubick, 1984). It was reported that rofecoxib, but not celecoxib, significantly increases systolic blood pressure in only 1 week in humans (Whelton, 2002). This might be due to a dysfunction of the elastin in arteries caused by rofecoxib.

From the above information and our present results, it is suggested that rofecoxib and/or its metabolite(s) are bound covalently to the elastin in the arteries, consequently leading to deteriorated elastin function. Considering the relationship between elastin and the diseases mentioned above, a long-lasting arterial dysfunction as a consequence of the covalent binding of rofecoxib may lead to

increased CV events, including possible hypertension in the short term. In our view, assuming that the same covalent binding observed in rats occurs in humans only for rofecoxib, the risk of CV events for CS-706 is thought to be less than that for rofecoxb and about the same as that for celecoxib. Further investigations regarding the mechanism of rofecoxib-elastin binding and the resulting toxicity are ongoing in our laboratories.

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Footnotes

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Legends for figures

Figure 1. Chemical structures of rofecoxib, celecoxib and CS-706

The asterisk indicates the position of the radiolabel.

Figure 2. Whole-body autoradioluminograms 2 or 48 h after single oral administration of [¹⁴C]COX-2 inhibitors to rats at a dose of 2 mg/kg (N=1/time point)

Figure 3. Whole-body autoradioluminograms after repeated oral administrations of [¹⁴C]rofecoxib to rats at a dose of 2 mg/kg (N=1/time point)

(A) 48 h after 3-day repeated administrations; (B) 48 h after 7-day repeated administrations; (C) 10 days after 7-day repeated administrations.

Figure 4. Radioactive concentrations in plasma and tissues after oral administration of [¹⁴C]COX-2 inhibitors to rats at a dose of 2 mg/kg (N=3, Mean ± SD)

(A, C and D) Single administration; Liver (open circle); Kidney (open triangle); Lung (open square); Plasma (open diamond); Aorta (closed circle); Auricular cartilage (closed triangle); and Skin (X).

(B) Single and multiple administrations of [¹⁴C]rofecoxib (once daily for 1, 3 or 7 days). P values

were calculated by Student's *t*-test (*, $p < 0.05$; **, $p < 0.01$).

Figure 5. Microautoradiograms showing the distribution of radioactivity 48 h after 7-day repeated administrations of [¹⁴C]rofecoxib to a rat at a dose of 2 mg/kg

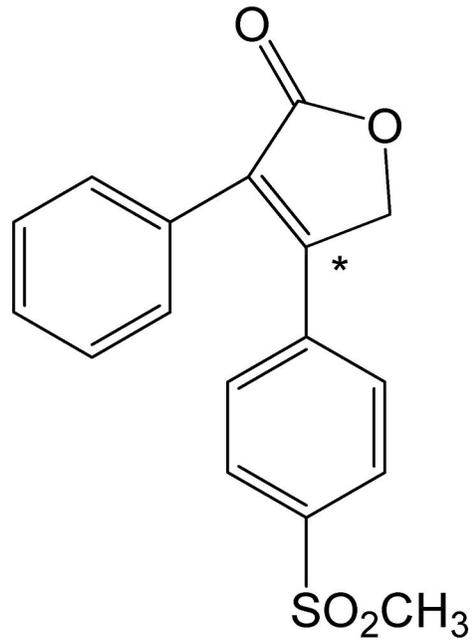
(A) Thoracic aorta, ×500; (B) Aortic arch, ×500; (C) Coronary artery, ×500. L, lumen; I, intima; M, media; A, adventia; El, elastic fiber.

Table 1. Enzymatic fractionation of covalently bound radioactivity in the thoracic aorta 24 h after single oral administration of [¹⁴C]rofecoxib to rats at a dose of 2 mg/kg. Covalently bound radioactivity (71.5% of total) in the aorta was fractionated by treatment with collagenase, elastase and pronase in series as described in *Materials and Methods*. In each step after enzyme treatment, the removable radioactivity from the tissue was measured.

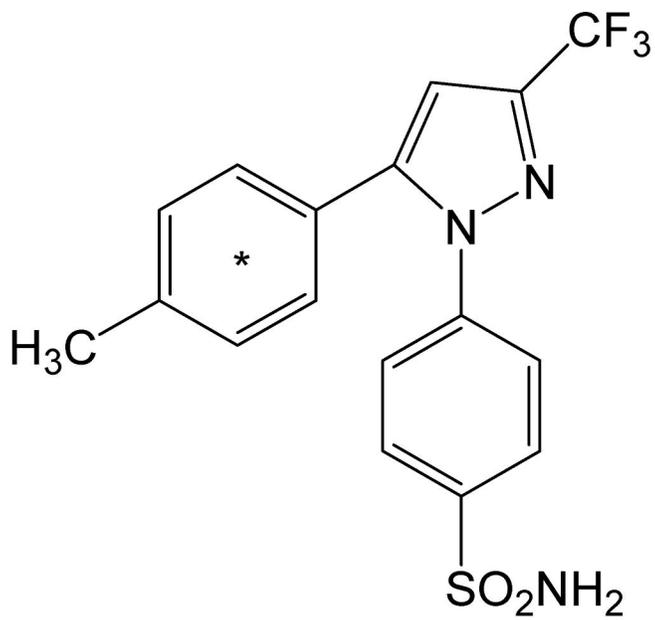
	Fraction			
	Collagenase	Elastase	Pronase	Residue
Radioactivity recovery (% of total covalent binding)	4.4	91.4	2.7	1.4

Figure 1

Rofecoxib



Celecoxib



CS-706

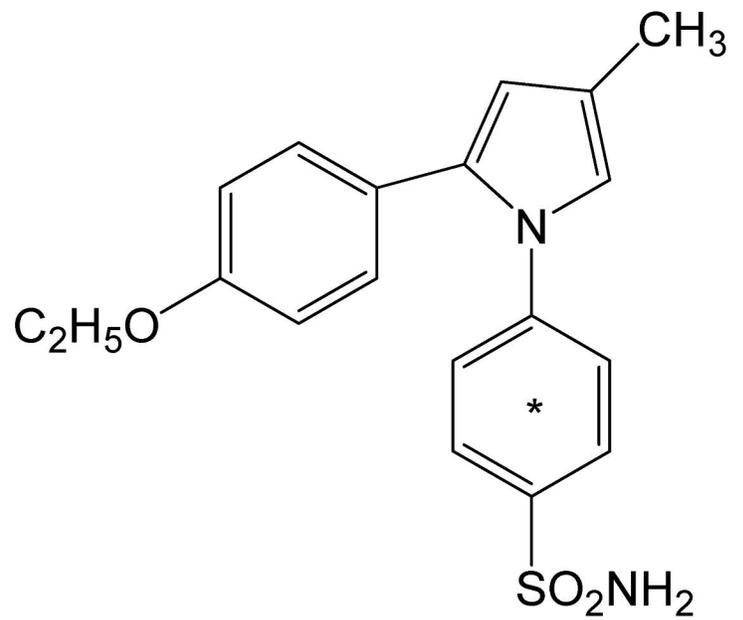
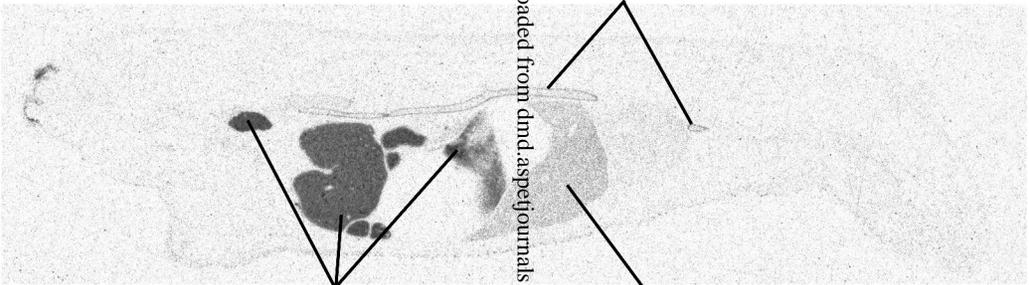
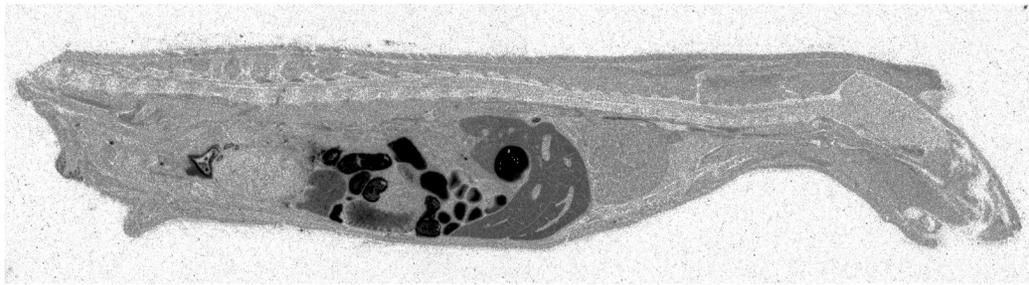


Figure 2

2 h

48 h

Rofecoxib

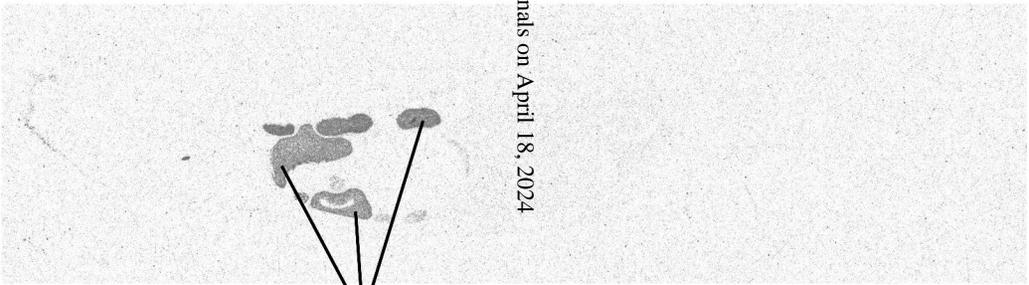
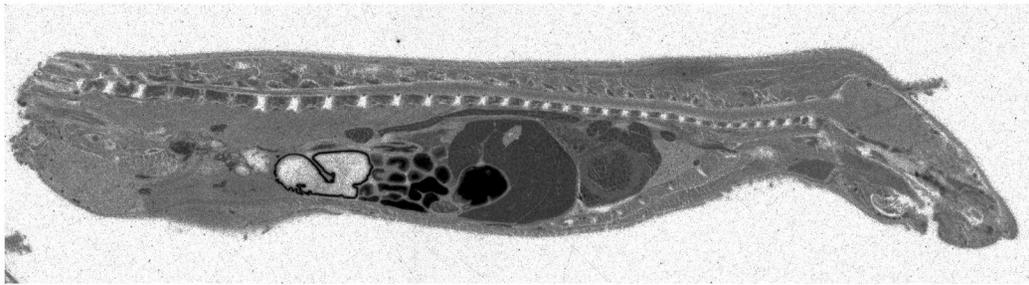


Gastrointestinal contents

Aorta

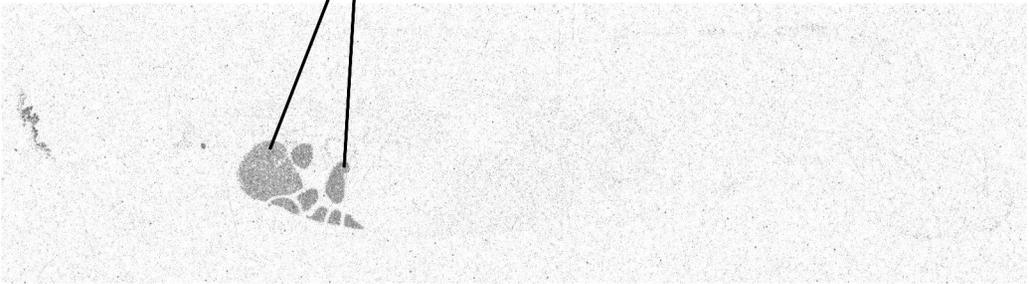
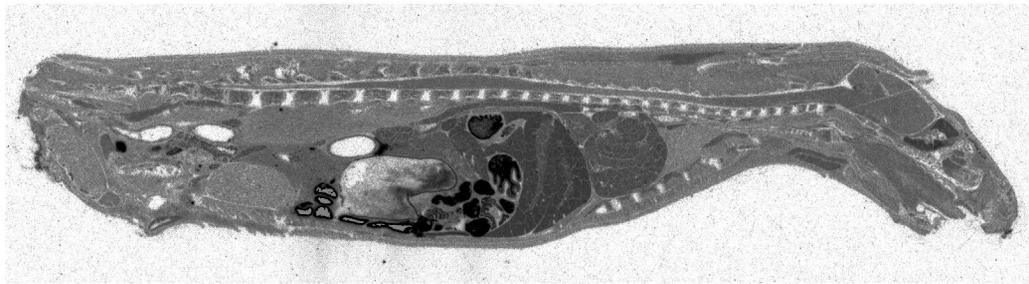
Liver

Celecoxib



Intestinal contents

CS-706



Low  High

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Figure 3

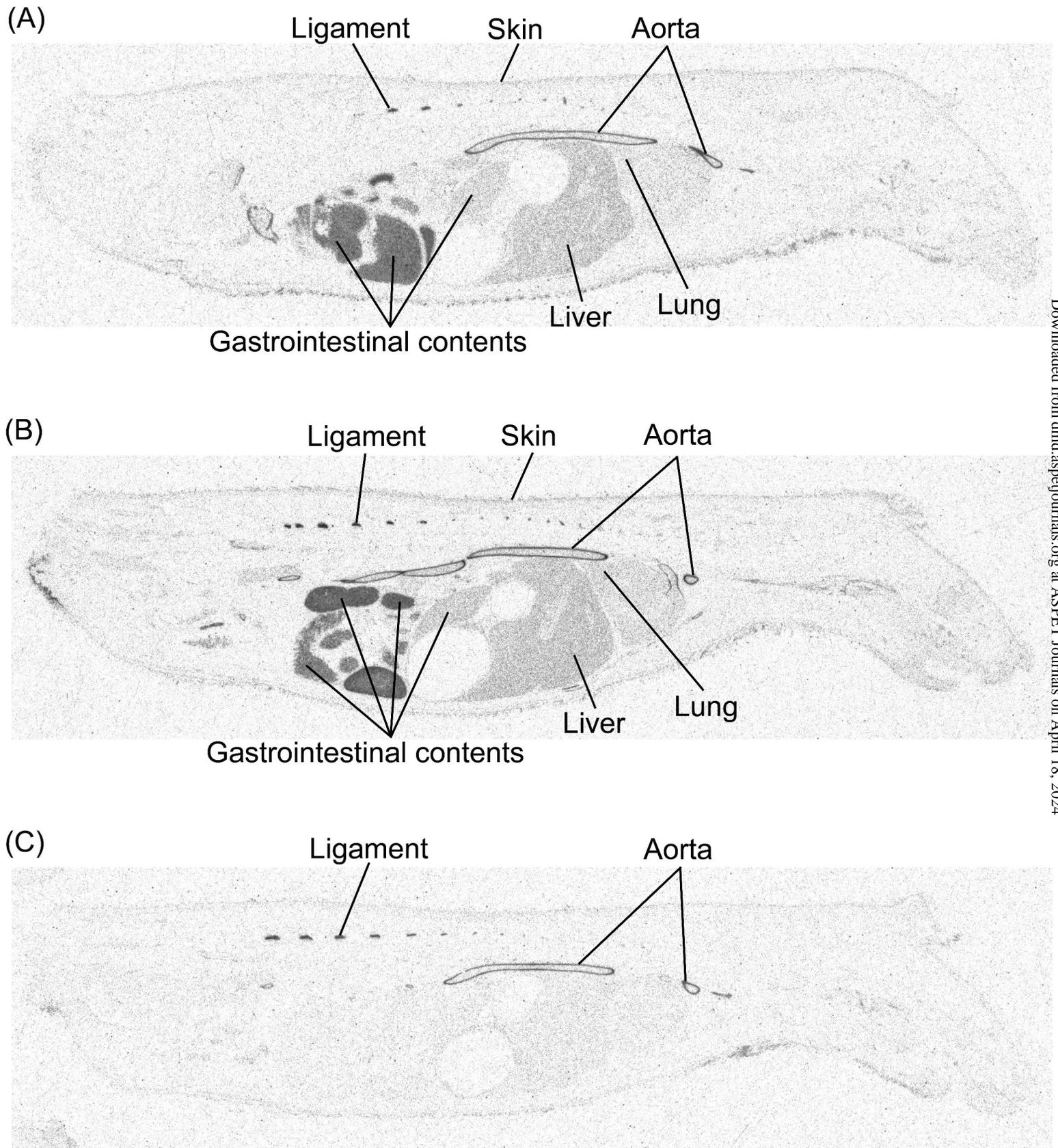
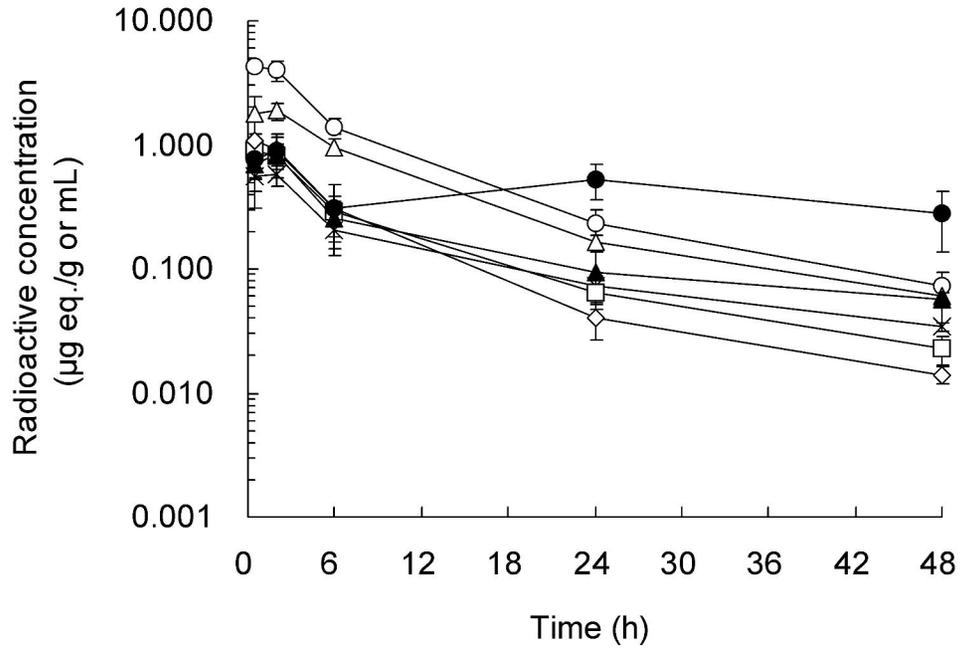
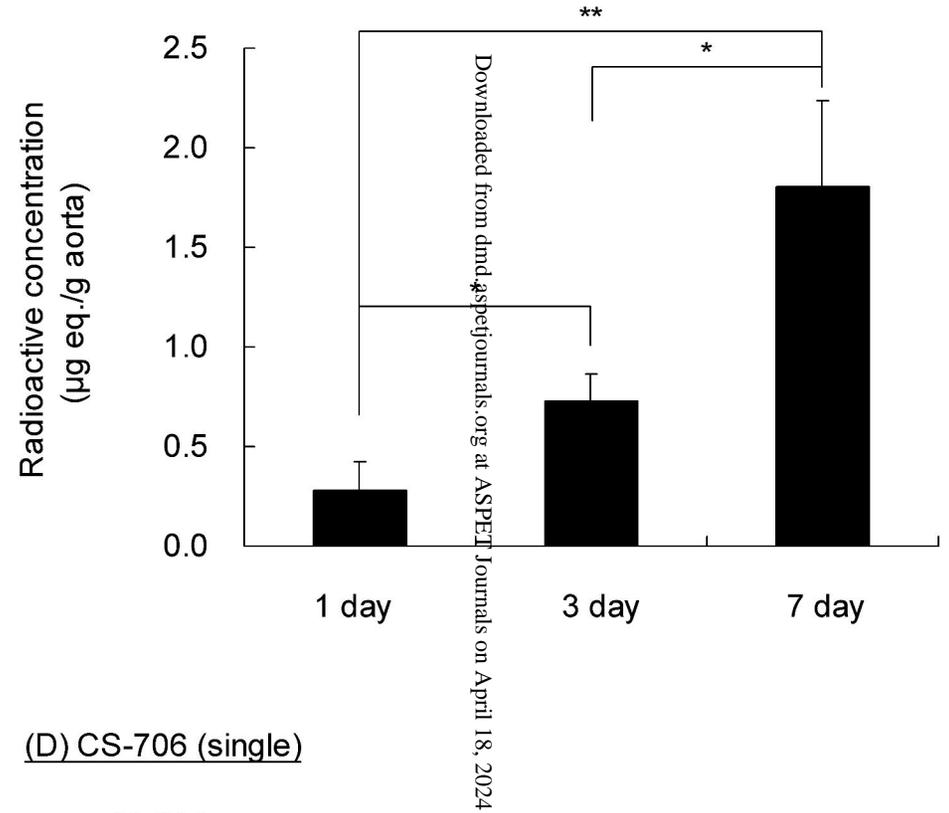


Figure 4

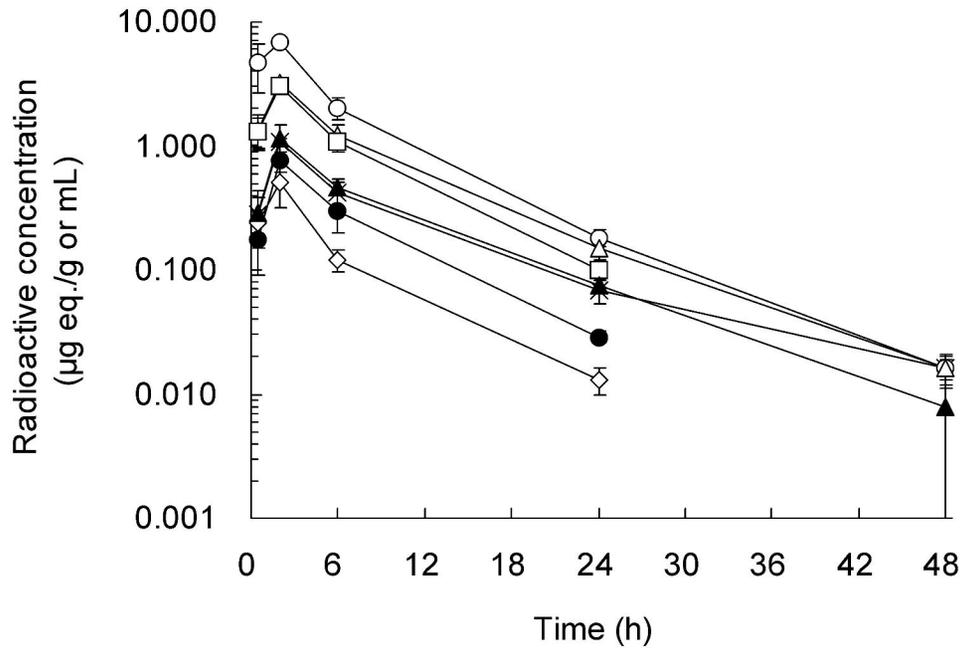
(A) Rofecoxib (single)



(B) Rofecoxib (single and multiple)



(C) Celecoxib (single)



(D) CS-706 (single)

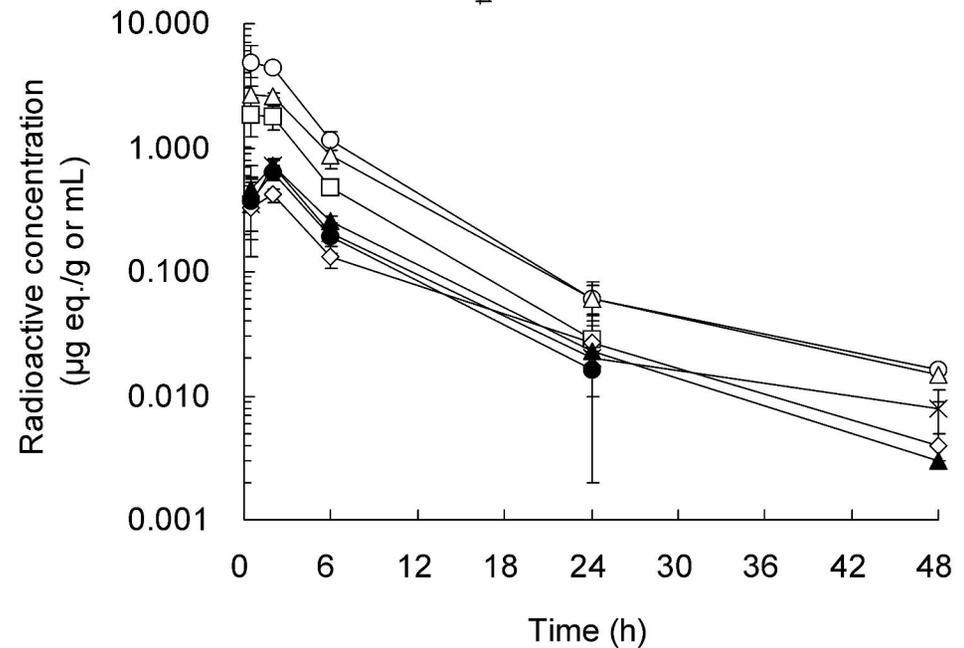


Figure 5

