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Pharmacological separation between peripheral and central functions of cyclooxygenase-2 with CIAA, a novel cyclooxygenase-2 inhibitor

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Abstract

There are many reports concerning the physiological and pathological involvement of cyclooxygenase (COX)-2 in the central nervous system and peripheral tissue cells. Selective COX-2 inhibitors that mainly distribute peripherally have not been reported thus far. Therefore central and peripheral roles of COX-2 remain classified pharmacologically. In this study, in vivo pharmacological profiles of CIAA ([6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-yl]acetic acid), a novel selective COX-2 inhibitor which distributes at higher concentrations in plasma than in brain, were compared with those of well-known selective COX-2 inhibitors, celecoxib and rofecoxib. Additionally, the possibility of pharmacological separation between peripheral and central actions of COX-2 with the inhibitors was investigated. CIAA selectively inhibited COX-2 activity compared with COX-1 in in vitro assays with rat whole blood. The compound exhibited lower brain penetration and higher plasma concentration (the brain/plasma concentration ratio was approximately 0.02) than celecoxib and rofecoxib after oral administration. Therefore, CIAA is mainly expected to act peripherally. Edema and prostaglandin E_2 (PGE_2) production in Carrageenan-injected rat paws, and pyrexia and PGE_2 production in the brain in lipopolysaccharide (LPS)-injected rats were measured in in vivo experiments. CIAA exhibited lower ratios of anti-pyretic/anti-edematous activities and of inhibitory activities of PGE_2 production in brain/paw than those of celecoxib and rofecoxib, and these ratios well-reflected brain/plasma concentration ratios. In conclusion, we discovered a novel selective COX-2 inhibitor, CIAA, which distributes at higher concentrations in plasma than in brain, which would make possible the pharmacological separation of the peripheral and central functions of COX-2.

Keywords: CIAA; Celecoxib; Rofecoxib; COX-2; Peripheral

1. Introduction

Prostaglandins, which are products of the COXs, play an important role in many physiological and pathological functions by acting on various kinds of prostaglandin receptors (DuBois et al., 1998; Narumiya et al., 1999). It is now well-established that COXs exist as two isoforms (Vane and Botting, 1995; Smith and Dewitt, 1996). The constitutive isoform, COX-1, is constitutively expressed in various tissues and is responsible for the production of prostaglandins involved in prostanoid-mediated physiological functions, such as gastric cytoprotection, maintenance of renal homeostasis and normal platelet functions (Crofford, 1997; Schafer, 1995). Although the inducible isoform, COX-2, has been identified and demonstrated to be highly expressed in inflammatory tissues (Xie et al., 1991; Masferrer et al., 1992; Hla and Neilson, 1992; Fu et al., 1990) and pyretic brain (Taniguchi et al., 1997), where it produces pro-inflammatory prostaglandins, it has been found to be constitutively expressed in some tissues including brain (Breder et al., 1995).

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ketrolac and indomethacin are nonselective COX

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inhibitors, demonstrating inhibitory activities in both COX-1 and COX-2. The therapeutic actions of these NSAIDs such as anti-inflammatory, analgesic and anti-pyretic effects can be explained by suppression of prostaglandin formations (Mitchell et al., 1994; Meade et al., 1993; Smith et al., 1994). On the other hand, it is well-known that NSAIDs show mechanism-based side effects, such as gastrointestinal and renal disorders (Patrono and Dunn, 1987; Sontag, 1986), leading to clinical limitation. Selective inhibitors of COX-2, such as celecoxib and rofecoxib, were recently developed as potential therapeutic agents and were expected to have anti-inflammatory and anti-pyretic effects similar to those of NSAIDs without the side effects via action on COX-1 (Gierse et al., 2005; Riendeau et al., 1997; Needleman and Isakson, 1997).

It was reported that PGE_2 or 6-keto-prostaglandin $F_{1\alpha}$ is produced by induced COX-2 in the tissue and infiltrated cells in carrageenan-induced inflamed sites, and that COX-2 selective inhibitors, such as NS-398 and SC-58125, suppressed plasma extravasation and prostaglandins synthesis induced by carrageenan (Masferrer et al., 1994; Seibert et al., 1994). On the other hand, it was reported that COX-2 was expressed and PGE_2 increased in yeast-induced febrile rat brain. Nimesulide, a selective COX-2 inhibitor, significantly suppressed febrile response and PGE_2 synthesis in brain (Taniguchi et al., 1997). However, so far, clear relationships between them have not been clarified because of the poor separated distribution in plasma and brain of the tested COX-2 inhibitors. If COX-2 inhibitors, which are mainly distributed peripherally or centrally, were discovered, a separation between peripheral and central functions of COX-2 would become possible pharmacologically.

We discovered CIAA ([6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-yl]acetic acid), a novel selective COX-2 inhibitor, which distributes at higher concentrations in plasma than in brain. In this study, the in vivo pharmacological profile of CIAA was compared with those of well-known selective COX-2 inhibitors, celecoxib and rofecoxib. Furthermore, the possibility of pharmacological separation between peripheral and central functions of COX-2 with the inhibitors was investigated. Edema and PGE_2 production in carrageenan-injected rat paws and pyrexia and PGE_2 production in the brain of LPS-injected rats were examined for peripheral and central functions, respectively, of the COX-2.

2. Materials and methods

2.1. Materials

Celecoxib, rofecoxib and CIAA ([6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-yl]acetic acid) were synthesized by Pfizer Global Research and Development (Aichi, Japan). λ-carrageenan (Picnin-A) was purchased from Zushikagaku Laboratories (Kanagawa, Japan). LPS from *E. coli* 0111:B4 and calcium ionophore A-23187 were purchased from Sigma (St. Louis, USA). Enzyme immunoassay (EIA) kit for PGE_2 or thromboxane B_2 (TXB_2) was purchased from Cayman Chemical Company (Ann Arbor, USA). PGE_2 [^{125}I]-radio immunoassay (RIA) kit was purchased from Amersham. Heparin sodium solution (Novo-Heparin, 1000 IU/ml) was purchased from Aventis Pharma (Tokyo, Japan). Methylcellulose (100 cp) was purchased from Wako chemical (Osaka, Japan).

2.2. Animals

All procedures used in in vivo assays were approved by the Animal Ethics Committee at the PGRD Nagoya Laboratories (Japan) according to the Laboratory Animal Welfare guidelines. Male Sprague–Dawley (SD) rats (135–150 g) were purchased from Charles River (Hino, Japan). Animals were housed in pairs in steel wire cages with free access to food and water. The animals were kept under conditions of constant temperature (23 ±2 °C) and humidity (55±15%) with a 12-h light/dark cycle (lights on 07:00 a.m.). Prior to the start of the experiment, the animals were housed under these conditions for 4–5 days. The rats were fasted for 16–18 h before experimental use. In the carrageenan-induced foot edema assay and LPS-induced pyrexia assay, the rats were orally given each drug suspended with 0.1% methylcellulose in a volume of 25 and 10 ml/kg, respectively.

2.3. Rat whole blood assay

The assay was conducted according to the procedures previously reported (Brideau et al., 1996). For the COX-2 assay, 80 μl of fresh heparinized rat whole blood was incubated with 10 μl of LPS (finally 30 μg/ml) or the vehicle (saline), and 10 μl of the test compound or the vehicle (1% dimethyl sulfoxide (DMSO); final 0.1%) for 24 h at 37 °C. The PGE_2 level in the plasma was measured by EIA according to the manufacturer's instructions. For the COX-1 assay, 80 μl of fresh heparinized rat whole blood was incubated with 10 μl of A-23187 (finally 30 μM) or the vehicle (saline), and 10 μl of the test compound or the vehicle (1% DMSO; final 0.1%) for 30 min at 37 °C. The TXB_2 level in the plasma was measured by EIA according to the manufacturer's instructions.

2.4. Carrageenan-induced paw edema

The carrageenan-induced rat paw edema assay was carried out using procedures previously described (Lombardino et al., 1975). Fasted male SD rats were orally given each test compound or the vehicle (0.1% methylcellulose in distilled water) 1 h before a carrageenan-injection. A line was drawn using a permanent marker on the ankle of the right hind paw to define the area of the paw to be monitored. The paw volume was measured just before the carageenan-injection using a plethysmometer (Unicom CO., Yachiyo, Japan). Then the rat was injected subplantarly with 100 μl of 1% (w/v) carrageenan solution or the vehicle (saline). The paw volume was measured 3 h after the carrageenan-injection and the change in paw volume during a 3 h period was calculated. In order to determine PGE_2 production in the inflamed paw, the rat was

euthanized and the foot was amputated just after measuring the paw volume 3 h after the carrageenan-injection and then immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.5. LPS induced pyrexia

After measuring the basal rectal temperature (at approximately 09:30 a.m.), the male SD rat was intraperitoneally given 0.5 mg/kg LPS or the vehicle (saline) in a volume of 5 ml/kg. Each test compound was orally administered 6 h after the LPS injection. The rectal temperature was measured 8 h after the LPS injection and the rectal temperature change during the 8 h period was calculated. In order to determine PGE_2 production in the brain, the rat was euthanized and the brain was removed just after measuring the rectal temperature 8 h after the LPS injection and then immediately frozen in liquid nitrogen and stored at −80 °C until the use.

2.6. Measurement of PGE_2 in rat paw and rat brain

The determination of PGE_2 in the tissues was carried out according to the method noted previously (Opas et al., 1987). The frozen foot was crushed, mixed with 7 ml of ethanol containing 10 μl/ml of indomethacin to inhibit PGE_2 production during sample handling, pulverized in a Waring blender and centrifuged at 2000×*g* for 10 min at 4 °C. The frozen brain was also mixed with 7 ml of ethanol containing 10 μl/ml of indomethacin, homogenized using HG30 Homogenizer (Hitachi, Tokyo, Japan) and clarified by centrifuge at 2000×*g* for 10 min at 4 °C. The PGE_2 in supernatants was extracted using a Sep-Pak C18 cartridge (Waters, Milford, USA) and dried in a vacuum. The sample was diluted to a final volume of 0.5 ml with assay buffer (PBS containing 0.1% w/v gelatin), and the PGE_2 level was determined by RIA according to the manufacturer's instructions.

2.7. Determination of plasma level of test compound

Plasma and brain concentrations of celecoxib, rofecoxib and CJ-018601 were determined by UV detection on HPLC (celecoxib: 250 nm, rofecoxib: 290 nm, CJ-018601: 330 nm, HP1090, Agilent, USA) on a Wakosil II5C18 HG (2×150 mm, Wako, Japan) after the extraction with acetonitrile. A gradient of 10% acetonitrile in 10 mM ammonium-acetate and 80% acetonitrile in 10 mM ammonium-acetate was used as the solvent system.

2.8. Data analysis

The data were presented as mean±S.E.M. The inhibitory activity of the test compound was shown as % inhibition when the values in groups with only stimulators and without any agents were expressed as 0% and 100% inhibition, respectively. IC_{50}, ID_{30} or ID_{50} values were calculated by computed linear regulation analysis. For comparisons between two groups, the data were analyzed using Student's *t*-test.

3. Results

3.1. In vitro COX-1 and COX-2 inhibitory activities

COX-1 and COX-2 in rat whole blood were activated by A-23187 and LPS stimulation, respectively, as described in Materials and methods. The inhibitory activities of celecoxib, rofecoxib and CIAA are shown in Table 1, and these compounds exhibited COX-2 inhibitory activities with IC_{50} values of 2.1, 0.70 and 1.3 μM, respectively. The three compounds inhibited COX-2 enzyme selectively as shown in Table 1.

3.2. Drug concentrations in plasma and brain

Celecoxib, rofecoxib and CIAA concentrations in plasma and brain were measured 1 h after oral administration at a dose of 10 mg/kg. The drug concentrations in plasma and brain are shown in Table 2. Rofecoxib and CIAA concentrations in plasma were 1.7- and 7.1-fold higher than that of celecoxib, respectively. On the other hand, rofecoxib and CIAA concentrations in brain were 2.2- and 14-fold lower than that of celecoxib, respectively. The brain/plasma ratios of the concentrations of these compounds were calculated as shown in Table 2. Brain/plasma ratios of celecoxib, rofecoxib and CIAA were 1.7, 0.48 and 0.018, respectively. CIAA showed higher peripheral selective distribution.

3.3. Effects on PGE_2 production in carrageenan-injected hind paw and LPS-treated rat brain

Fig. 1A and B shows the effects of celecoxib, rofecoxib and CIAA on PGE_2 production in carrageenan-injected rat hind paw and LPS-injected rat brain, respectively. Each compound was orally administered 1 h before carrageenan or 5 h after LPS injection. The inflamed paw or brain was removed 3 h after carrageenan injection or 7 h after LPS injection, respectively, and the PGE_2 content was quantified using an RIA assay kit. The PGE_2 content in carrageenan-injected rat paw (1400±20 ng/paw) or LPS-injected rat brain (17,000±2000 ng/brain) was significantly higher than that in each vehicle-injected rat paw (320±20 ng/paw) or brain (2400±300 ng/brain), respectively. All four compounds inhibited PGE_2 synthesis in both tissues in a dose-dependent manner, and the maximum efficacies reached 100% inhibition. The ID_{50} values of celecoxib, rofecoxib and CIAA were 2.6, 1.0 and 2.9 mg/kg, p.o., respectively, in carrageenan-injected paw, and 0.14,

Table 1
In vitro rat COXs inhibitory activities of celecoxib, rofecoxib and CIAA

	COX-1 IC50 (uM)	COX-2 IC50 (uM)	Selectivity
Celecoxib	46±3	2.1±0.8	22
Rofecoxib	72±16	0.7±0.2	100
CIAA	31±11	1.3±0.3	23

COX-1 and COX-2 activities were analyzed by measuring A-23187-stimulated TXB_2 production and LPS-stimulated PGE_2 production from rat whole blood, respectively. Values are expressed as means±S.E.M for all three experiments.

Table 2
Drug concentrations in plasma and brain

Compound	Plasma (μg/ml)	Brain (μg/g)	Brain/plasma ratio
Celecoxib	1.1±0.2	1.9±0.2	1.7
Rofecoxib	1.9±0.3	0.89±0.18	0.47
CIAA	7.8±1.2	0.14±0.03	0.018

Plasma and brain were taken 1 h after drug administration and drug concentrations were measured by HPLC or LC-MS after extraction of the drug. Values are expressed as means±S.E.M of plasma and brain concentrations for three rats.

0.65 and 14 mg/kg, p.o., respectively, in LPS-injected brain (Table 3).

3.4. Effects on carrageenan-induced paw edema and LPS-induced pyrexia

Fig. 2 presents the anti-inflammatory effects of celecoxib, rofecoxib and CIAA on carrageenan-induced paw edema. Significant paw edema was observed 3 h after a carrageenan-injection (the paw volume changes after carrageenan injection and after vehicle injection were 0.62±0.04 ml and −0.016± 0.014 ml, respectively). When administered 1 h before the injection of carrageenan, celecoxib, rofecoxib and CIAA inhibited paw edema in a dose-dependent manner. Maximum efficacies of these compounds reached approximately 60%

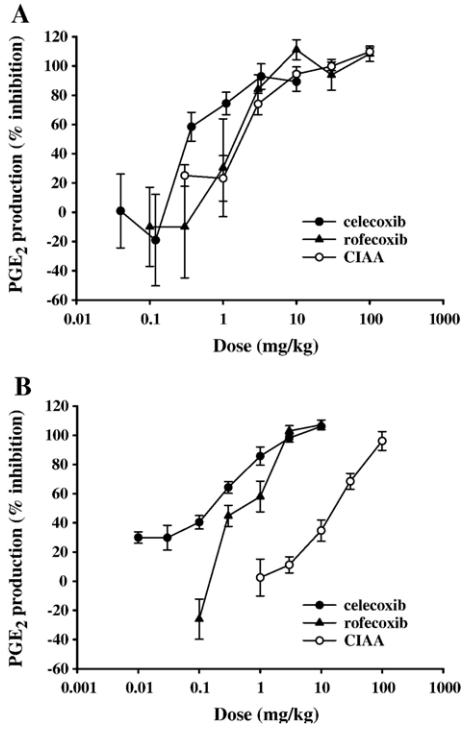

Fig. 1. Inhibitory effects of celecoxib, rofecoxib and CIAA on PGE_2 production in (A) carrageenan-injected rat paw and (B) LPS-treated rat brain. Each drug was orally administered 1 h before intraplantar carrageenan (1 mg/paw) injection or 6 h after intraperitoneal LPS (0.5 mg/kg) injection. The rat was euthanized 3 h after carrageenan injection or 8 h after LPS injection, and the inflamed paw and the brain were taken, respectively. The PGE_2 level was determined by RIA. Data were expressed means±S.E.M for 6–7 rats.

Table 3
Pharmacological profiles of celecoxib, rofecoxib and CIAA

	Pyrexia ID_{50} (mg/kg)	Edema ID_{30} (mg/kg)	Brain PGE_2 ID_{50} (mg/kg)	Paw PGE_2 ID_{50} (mg/kg)	Ratio of activity (central/peripheral)	
					A	B
Celecoxib	0.45	2.5	0.14	2.6	5.5	26
Rofecoxib	0.61	3.4	0.65	1.0	5.6	1.6
CIAA	35	3.8	14	2.9	0.11	0.21

ID_{30} or ID_{50} value was calculated using value of % inhibition at each dose by computed linear regulation analysis. *A*: The ratio of central activity to peripheral activity of each compound against pyrexia and edema was calculated as {1/(ID_{50} value of pyrexia)}/{1/(ID_{30} value of edema)}. *B*: The ratio of central activity to peripheral activity of each compound against in brain PGE_2 production and in paw PGE_2 production was calculated as {1/(ID_{50} value of brain PGE_2)}/{1/(ID_{50} value of paw PGE_2)}.

inhibition, so ID_{30} values were calculated. ID_{30} values of celecoxib, rofecoxib and CIAA were 2.5, 3.4 and 3.8 mg/kg, p.o., respectively.

Fig. 3 presents the anti-pyretic effects of celecoxib, rofecoxib and CIAA in LPS-injected rats. Rectal temperature significantly increased by 2.0±0.1 °C 8 h after the LPS injection (saline injection: −0.12±0.11 °C). Celecoxib, rofecoxib and CIAA were administered orally 5 h after LPS injection. The anti-pyretic effects of these compounds were evaluated 2 h after the drug administration. Celecoxib, rofecoxib and CIAA exhibited anti-pyretic effects in a dose-dependent manner with ID_{50} values of 0.45, 0.61 and 35 mg/kg, p.o., respectively (Table 3).

4. Discussion

It has been reported that an induced COX-2 plays an important role in both physiological and pathological states, including inflammation, angiogenesis, bone absorption, colon cancer, hyperalgesia, Alzheimer's disease and so on (Katori and Majima, 2000). The relationship between COX-2 expression and subsequent prostaglandins production with induction of inflammation has been well investigated in animal models. In the rat carrageenan air pouch model, vasodilation and edema

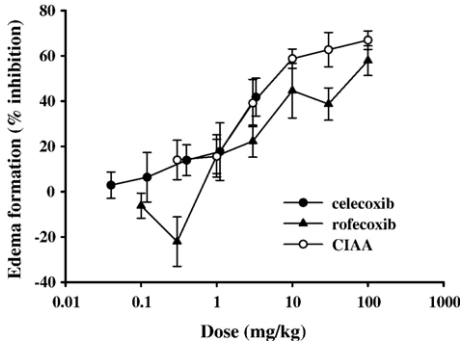

Fig. 2. Inhibitory effects of celecoxib, rofecoxib and CIAA on carrageenan-induced paw edema in rats. The rat received intraplantar injection of carrageenan (1 mg/paw) 1 h after oral administration of each compound. Paw volume was measured before and 3 h after carrageenan injection. Data were expressed means±S.E.M for 6–7 rats.

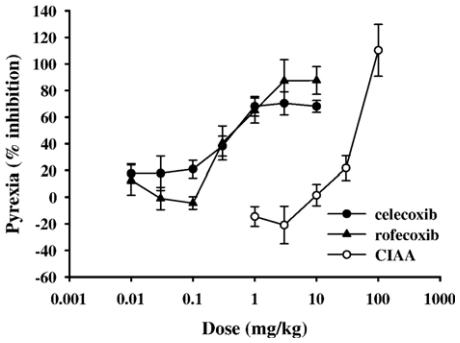

Fig. 3. Inhibitory effects of celecoxib, rofecoxib and CIAA on LPS-induced pyrexia in rats. Rats received an intraperitoneal injection of LPS (0.5 mg/kg) 6 h before oral administration of the test compounds. The rectal temperature was measured before and 8 h after LPS injection. Data were expressed means±S.E.M. for 6–7 rats.

was induced 6 h after the carrageenan injection, and upregulation of COX-2 mRNA and protein coincided with the production of prostaglandins in the peripheral inflammatory tissues (Masferrer et al., 1994). Furthermore, the selective neutralization antibody of PGE_2 suppressed edema formation and hyperalgesia in carrageenan injected rats (Portanova et al., 1996). In the LPS pyresis model, COX-2 mRNA was induced in the rat brain in parallel with febrile response after intraperitoneal injection of LPS (Cao et al., 1997), and the fever was suppressed by microinjection of ketrolac into the anteroventral preoptic region (Scammell et al., 1998). Intracerebroventricularly (i.c.v.) injected PGE_2 produced fever in wild type mice. On the other hand, i.c.v. injected PGE_2 did not produce fever in EP_3 knock out mice (EP_3 is one subtype of PGE_2 receptors) (Ushikubi et al., 1998). Immuno-reactive COX-2 was induced in perivascular microglia along the cerebral blood vessels and in meningeal macrophages at the edge of the brain in febrile rats (Schiltz and Sawchenko, 2003; Elmquist et al., 1997). Thus it may be suggested that PGE_2 generated by induced COX-2 in inflammatory states acts separately in peripheral tissues and in the central nervous system. However, selective COX-2 inhibitors such as L-745377 and rofecoxib suppress carrageenan-induced edema and LPS-induced pyrexia in rats at the same dose range (Chan et al., 1995, 1999). This is due to the poor separated distribution in plasma and brain of the tested COX-2 inhibitors. COX-2 inhibitors with separated distribution in plasma and brain would pharmacologically help separation of the peripheral and central functions of COX-2. We discovered a novel COX-2 selective inhibitor, CIAA, which had a comparable potency to COX-2 and the COX-2/COX-1 selectivity of celecoxib. The brain/plasma ratios of celecoxib, rofecoxib and CIAA after oral administration at 10 mg/kg were 1.71, 0.48 and 0.018, respectively. Thus CIAA showed mainly peripheral distribution, whereas celecoxib and rofecoxib showed almost the same distribution level between plasma and brain, and CIAA may be a special pharmacological tool to separate peripheral and central functions of COX-2.

In vivo pharmacological profiles of CIAA in comparison with celecoxib and rofecoxib were investigated in rat inflammatory models. Edema and PGE_2 production in carrageenan-injected rat paw, and pyrexia and PGE_2 production in brain in LPS-injected rats were examined for peripheral and central functions, respectively, of COX-2. Celecoxib, rofecoxib and CIAA inhibited foot edema and PGE_2 production in paw after carrageenan injection in a dose dependent manner. The ID_{30} values against foot edema of these compounds were 2.5, 3.4 and 3.8 mg/kg, p.o., respectively, and the ID_{50} values against PGE_2 production in paw were 2.6, 1.0 and 2.9 mg/kg, p.o., respectively. The ID_{30} values against foot edema and the ID_{50} values against PGE_2 synthesis were well correlated. On the other hand, celecoxib, rofecoxib and CIAA suppressed pyrexia and PGE_2 production in brain after LPS treatment in a dose dependent manner. The ID_{50} values against pyrexia of these compounds were 0.45, 0.61 and 35 mg/kg, p.o., respectively and the ID_{50} values against PGE_2 production in brain were 0.10, 0.60 and 14 mg/kg, p.o., respectively. The ID_{50} values against pyrexia and the ID_{50} values of PGE_2 synthesis were well correlated. Thus the pharmacological data demonstrated that peripheral and central PGE_2 generated by COX-2 were important mediators in carrageenan-induced inflammation and LPS-induced pyrexia, respectively.

The relationship between central function/peripheral function ratios of celecoxib, rofecoxib and CIAA and their brain/plasma distribution ratios were analyzed in order to show pharmacological separation between peripheral and central functions of COX-2. As shown in Table 3, the ratios between ID_{50} values against pyrexia and ID_{30} values against edema of celecoxib, rofecoxib and CIAA were 5.6, 5.6 and 0.11, respectively. The ratios of rofecoxib and CIAA normalized with the ratio of celecoxib were 1.0 and 0.020, respectively. On the other hand, the ratios between ID_{50} values against brain PGE_2 production and ID_{50} values against paw PGE_2 production of celecoxib, rofecoxib and CIAA were 26, 1.6 and 0.21, respectively. The ratios of rofecoxib and CIAA normalized with the ratio of celecoxib were 0.062 and 0.0081, respectively. The brain/plasma ratios of rofecoxib and CIAA normalized with the brain/plasma ratio of celecoxib were calculated as 0.28 and 0.012, respectively. These data, which show a close relationship between central function/peripheral function ratios of celecoxib, rofecoxib and CIAA and their brain/plasma ratios, can indicate pharmacological separation between peripheral and central functions of COX-2 and CIAA, which is a beneficial tool for the investigation.

In conclusion, we discovered a novel selective COX-2 inhibitor, CIAA, which distributes at higher concentrations in plasma than in brain. It became possible to separate peripheral and central functions of COX-2 pharmacologically using CIAA. This compound is a useful tool to investigate the pathophysiological role of COX-2 in peripheral tissue and organs.

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