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CC05, a novel anti-inflammatory compound, exerts its effect by inhibition of cyclooxygenase-2 activity

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Abstract

In the present study, we examined the anti-inflammation of a novel compound, 4-[5-(3-amino-4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (CC05) in vitro and in vivo. In an in vitro cell-based assay, CC05 inhibited cyclooxygenase (*COX*)-*2*-derived prostaglandin E_2 (PGE_2) synthesis with an IC_{50} value of 0.328 ± 0.04 μM compared with an IC_{50} value of 14.34 ± 0.05 μM for the inhibition of *COX-1*-derived PGE_2 synthesis. In two in vivo rodent models, CC05 (12.5, 25 and 50 mg/kg, i.g.) is a moderate potential and selective inhibitor of *COX-2*. It can reduce carrageenan-induced paw edema and PGE_2 production in the inflamed pouch dose-dependently without affecting the PGE_2 production in stomachs. Furthermore, CC05 had no effect on *COX-2* mRNA and protein expression in phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 human macrophages stimulated with lipopolysaccharide (LPS). These results demonstrate that CC05 is a novel *COX-2* inhibitor and the anti-inflammatory action is not directed towards the transcription or translation of the *COX-2* gene but only to the enzymatic activity of the protein.

Keywords: Cyclooxygenase-2; Inflammation; Carrageenan-induced paw edema; Air pouch model; THP-1 cell line

1. Introduction

Cyclooxygenase (COX) is a key enzyme regulating the formation of prostaglandins (PGs) from arachidonic acid. COXs are also major therapeutic targets for nonsteroidal anti-inflammatory drugs (NSAIDs; Guan et al., 1997; Harris et al., 1994; Smith and Wilkin, 1977). Two isoforms of COX, designated *COX-1* and *COX-2*, have been identified (DeWitt and Smith, 1988; Xie et al., 1991). These derive from different genes but share ~60% amino acid identity (Hla and Maciag, 1991; Kujubu et al., 1991). The expression pattern of *COX-1* and *COX-2* genes is quite different. *COX-1* is constitutively expressed in several tissues and is thought to participate in housekeeping functions, including the maintenance of gastric epithelial integrity. Conversely, *COX-2* is undetectable in most tissues, but its expression can be induced by a variety of cytokines and growth factors (Herschman, 1996; Hla and Neilson, 1992; Vane and Botting, 1998). It is reported that a specific *COX-2* inhibitor will achieve therapeutic efficacy in acute and chronic inflammation management while avoiding the serious side effects, in particular, gastrointestinal ulceration related to *COX-1* inhibition observed with NSAIDs (Prasit et al., 1999; Hawkey, 1999; Mitchell and Warner, 1999). A number of useful and selective *COX-2* inhibitors such as rofecoxib and celecoxib were introduced on the market (Prasit et al., 1999; Penning et al., 1997). However, the increased incidence of nongastrointestinal serious adverse events, with the *COX-2* selective inhibitors as compared with nonselective NSAIDs, in the Celecoxib Long-term Arthritis Safety Study (CLASS) and the Vioxx Gastrointestinal Outcomes Research (VIGOR) study remains a major concern (Wright, 2002). With all these aspects considered, developing drugs that preferentially inhibit *COX-2* with moderate potency and selectivity may be more promising.

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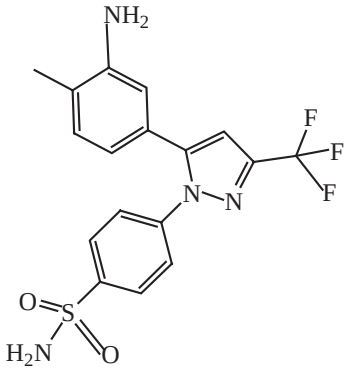

Fig. 1. Chemical structure of 4-[5-(3-amino-4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulfonamide (CC05).

Benzenesulfonamide derivatives exhibit anti-inflammatory activity in experimental models and they exert their action by inhibition of *COX-2* (Hashimoto et al., 2002). In the present work, we reported for the first time the anti-inflammatory activity of CC05 (Fig. 1), a novel benzenesulfonamide compound structurally similar with celecoxib, on acute and sub-acute inflammation after intragastric (i.g.) administration in rats. Furthermore, we also examined whether CC05 can inhibit *COX-2* expression in phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 human macrophages stimulated with lipopolysaccharide (LPS) at mRNA and protein levels.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 180–200 g, were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences. All animals were housed in Plexiglas cages and kept on a 12/12 h light–dark cycle in temperature and humidity controlled rooms. Food was withheld 12 h before the experiments, with free access to water. Unless otherwise indicated in the text, standard laboratory food and water were provided ad libitum. Experiments were performed between 9:00–17:00 h. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Cell culture and treatment

Insect cell line *Spodoptera frugiperda* (Sf9), obtained from the Institutes of Biochemistry and Cell Biology, Shanghai Institutes for Biological Science, Chinese Academy of Sciences, was cultured in monolayer at 28 °C in Grace's supplemented medium (Gibco BRL) with 10% heat-inactivated fetal calf serum (Gibco BRL).

The human monocytic cell line THP-1 was purchased from American Type Culture Collection (ATCC) and cultured in complete RPMI-1640 medium (Gibco/BRL Life Technologies) supplemented with L-glutamine, 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate. Cells were kept at 37 °C in 5% CO_2 and harvested at the logarithmic phase of growth. Then cells were adjusted to 1×10^6/ml in the presence of 10 nM PMA (Sigma) and incubated for 48 h to differentiate into adherent macrophages. The cells were quiesced for 24 h by incubation in fresh RPMI-1640 complete medium before the experiments. LPS (1 μg/ml) was used as a standard concentration for stimulation according to Barrios-Rodiles et al. (1996) with modification. Macrophages were pretreated for 30 min with the tested drugs and then stimulated with LPS for 4 h in the presence of the drugs.

2.3. In vitro cell-based assay of COX inhibition

Cell-based assay was performed as previously described (Zhang et al., 2004). Briefly, 24 h after infecting Sf9 cells with h*COX-1* or h*COX-2* recombinant baculovirus, the cells were collected and washed in Hank's solution buffered with 15 mM HEPES (HHBS, pH 7.4) adjusted to 1×10^6/ml, placed in 24-well plates and incubated for 15-min with drugs or vehicles at 37 °C. Then cells were challenged with 10 μM arachidonic acid (Sigma) in ethanol and incubated for 10 min. The cells were pelleted for 10 min at 300 $\times g$ and the levels of prostaglandin E_2 (PGE_2) in the supernatant were determined by a PGE_2-specific radioimmunoassay (RIA). The concentration of PGE_2 was then determined by interpolation from a standard curve and inhibition calculated by comparison of the PGE_2 production by drug-treated cells with that of dimethylsulphoxide (DMSO)-treated cells.

2.4. In vivo tests of inflammation

2.4.1. Carrageenan-induced paw edema

The inflammatory response was induced by sub-plantar injection of 100 μl of 1% (w/v) sterile carrageenan in saline into the right hind paw as described previously (Winter et al., 1962). The volume of the injected paw was measured with a plethysmometer (Shandong Academy of Medical Sciences, China) before and 3 h after the injection. The inflammation index was calculated as the difference between the final volume of the carrageenan injected paw (V_t) and the initial volume of the same paw before injecting it (V_0), i.e., Inflammation index (I_i) = $V_t - V_0$. The edema inhibition (%) was calculated as the percentage of the difference of I_i according to the following formula: % Inhibition = [(pre-drug I_i) − (post-drug I_i)] / (pre-drug I_i) × 100. CC05 (12.5, 25 and 50 mg/kg) were given intragastrically 12 h before carrageenan challenged. Celecoxib (30 mg/kg, i.g.) only reduced rats paw edema by 30% (Francischi et al., 2002), thus we choose 50 mg/kg (i.g.) in this study. Celecoxib and indomethacin (10 mg/kg, i.g.) were administered 0.5 h before carrageenan injected. Rats of the control group received the same volume of saline according to their weights.

2.4.2. Air pouch model of inflammation

Air pouch was produced by subcutaneous injection of 20 ml sterile air into the intrascapular area of the back as previously described (Sedgwick et al., 1983; Sedgwick and Lees, 1986). One day after initial air injection, carrageenan (0.5%, 5 ml) was injected directly into the pouch using a 5-ml syringe and a 20-G, 1-in needle to induce inflammation. After 3 h of carrageenan treatment, 10 ml of 5.4 mM EDTA disodium was injected into the air pouch of each rat using a 10-ml syringe and 18-G, 1.5-in needle. Mix the contents of the pouch by gently massaging the area. Then animals were euthanized by CO_2 asphyxiation and the pouch fluid was collected for PGE_2 determination by RIA (Moroney et al., 1988). The stomachs of these animals were excised, opened, and cleaned, and the mucosal lining was dissected, weighed and frozen in liquid nitrogen. Stomach tissue was processed by homogenization with 0.9% saline and absolute alcohol (1:4). After centrifuged, the supernatants were stored at −30 °C for PGE_2 determination by RIA. The dose and administration protocols of drugs were the same as those of the *paw edema test*.

2.5. RNA extraction and reverse transcription

Total RNA was extracted from THP-1 cells using TRIzol (Invitrogen, Inc) according to the manufacturer's protocol. The RNA concentration was determined spectrophometrically. Two microgram of RNA was reversely transcribed in a volume of 20 μl at 42 °C for 60 min (all chemicals were obtained from Promega).

2.6. Real time polymerase chain reaction (PCR)

Real time PCR detects the accumulation of PCR product at every cycle of amplification via optics- and imaging-system, which recorded the binding of a fluorescent dye (SYBR Green) to double-stranded DNA (Heid et al., 1996). Relative quantification of changes in *COX-2* mRNA expression was determined using real time PCR analysis, with β-actin as the endogenous control. Specific primers designed were shown in Table 1. The real time PCR was carried out with the following profile using *Taq* DNA polymerase (Takara Biotech): 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 45 s, 60 °C for 60 s. To proved that the cDNA of *COX-2* and β-actin were amplified with the same efficacy, a standard curve was made where the Ct values were plotted against cDNA concentration based on the equation: $Ct = \log(\text{concentration}/(1+E))$, where E is the amplification efficiency with the optimal value of 1. To prove that each primer-pair generates only one PCR product, an agarose gel electrophoresis with the PCR products was run (data not shown). Changes in *COX-2* mRNA were then quantified by real time PCR and the ΔΔCt method (Heid et al., 1996).

Table 1
Sequences of primers used for real time PCR analysis

		Sequence
COX-2	Forward primer	5′-TTCAAATGAGATTGTGGGAAAATTGCT-3′
	Reverse primer	5′-AGATCATCTCTGCCTGAGTATCTT-3′
β-actin	Forward primer	5′-AGCGGGAAATCGTGCGTG-3′
	Reverse primer	5′-CAGGGTACATGGTGGTGCC-3′

2.7. Western blot analysis

COX-2 protein expression was studied in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. THP-1 cells were grown in 10 cm cell culture dishes, and differentiated with PMA (10 nM). At the indicated time-points, pelleted cells were harvested with 0.02% typsin/EDTA and washed with PBS twice (4 °C, pH 7.4), resuspended in 100 μl ice-cold cell lysis buffer (Tris 10 mM pH 7.5, NaCl 130 mM, Triton X-100 1%, NaF 1 mM, sodium orthophosphate (NaPi) 10 mM, sodium pyrophosphate (NaPPi) 10 mM, phenylmethylsulphonyl fluoride (PMSF) 1 mM) containing protease inhibitor cocktail (aprotinin 5 μg/ml, leupeptin 5 μg/ml, pepstatin A 5 μg/ml), and lysed cells on ice for 20 min. The lysate was centrifuged at 4000 ×*g* for 15 min. Protein concentrations were determined by BCA Protein Assay Kit (Pierce). Whole cell lysates were mixed with 2 × SDS sample buffer and heated to 100 °C for 5 min. Samples, containing 25 μg protein, were electrophoresed in 8% SDS-PAGE and separated proteins were transferred on to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked at 4–8 °C for overnight with 5% non-fat dried milk in Tris-buffered saline with Tween 20 (0.1%) and incubated at room temperature for 1 h with monoclonal anti-β-actin (Sigma) or goat polyclonal IgG anti-*COX-2* antibodies (Santa Cruz Biotechnology). The appropriate secondary horseradish peroxidase-conjugated antibody (DAKO) was added and complexes were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech).

2.8. Drugs

Carrageenan (Sigma, St. Louis, MO, USA) was suspended in saline. CC05, 4-[5-(3-amino-4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide and celecoxib were kindly provided by Professor Jing-Kang Shen (Shanghai Institute of Materia Medica, Chinese Academy of Sciences). CC05, celecoxib and indomethacin (Sigma) were dissolved in 0.5% carboxymethylcellulose immediately before experiments and administered intragastrically. PGE_2-specific RIA kits was purchased from Beijing East Asia Institute of Immunology. PMA (Sigma) was dissolved with DMSO and diluted with fresh RPMI-1640 medium before the experiments (1:1000). Dexamethasone and LPS (*Escherichia coli*, strotype 0111:B4) were products from Sigma (St. Louis, MO, USA).

2.9. Data analysis

All data were expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) with Dunnett's post hoc analysis was used to determine whether the individual doses produced significance. The real time PCR results were compared using two-way repeated measures ANOVA. A value of $P<0.05$ was considered significant in this study.

3. Results

3.1. Effect of CC05 on COX-1 and/or COX-2 enzymatic activity in vitro

The effect of CC05 on enzymatic activity of COX, indirectly measured by the amount to PGE_2 produced, was evaluated in intact infected Sf9 cells expressing comparable amounts of recombinant h*COX-1* or h*COX-2*. Inhibition profiles of CC05, indomethacin (preferentially *COX-1* inhibitor) and celecoxib (*COX-2* selective inhibitor), obtained from this cell-based assay system, were illustrated the magnitude of the differences in IC_{50} values (Table 2). Although the selective ratio ($IC_{50,\ COX\text{-}2}/IC_{50,\ COX\text{-}1}$) of CC05 was 0.0229, similar to that of celecoxib (0.0221), IC_{50} values of CC05 to both *COX-2* and *COX-1* were approximately 6-fold larger than those of celecoxib.

3.2. Effect of CC05 on carrageenan-induced paw edema

In vivo, CC05 (12.5, 25 and 50 mg/kg, i.g.) can inhibit the inflammatory edema dose-dependently. The reductions of paw edema were 8.96%, 40.38% and 71.33%, respectively. Indomethacin (10 mg/kg, i.g.) reduced the paw edema by 76.36% and celecoxib (50 mg/kg, i.g.) only reduced by 44.89% (Fig. 2).

3.3. Effect of CC05 on carrageenan-induced inflammation in air pouch model

The selective inhibition of CC05 on *COX-2* in vivo was assessed in carrageenan-induced air pouch model. This model was chosen because the inflammatory response includes the production of large amounts of PGE_2 derived from expression of *COX-2* (Masferrer et al., 1994). Like the paw edema induced by carrageenan, injection of carrageenan into an established subcutaneous air pouch in rats induced marked PGE_2 production. PGE_2, produced from air pouch were inhibited by CC05 in a dose-dependent manner, and CC05 at a dose of 50 mg/kg produced a significant reduction. However, PGE_2, produced from the stomach were not inhibited by CC05 at any dose. The inhibition manner was different from indomethacin, similar to that of celecoxib, which could inhibit the production of PGE_2 driven either from the air pouch or the stomach (Fig. 3).

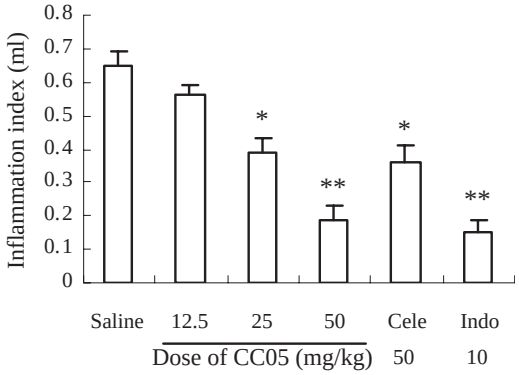

Fig. 2. Effect of CC05 on carrageenan-induced paw edema. CC05 (12.5, 25 and 50 mg/kg, i.g.) or saline were administered 12 h before initiation of inflammation with carrageenan. Celecoxib (50 mg/kg, i.g.) and indometacin (10 mg/kg, i.g.) were administered 0.5 h before carrageenan injection. Edema was measured 3 h after carrageenan injection. Each column presents as mean± S.E.M. ($n=6-8$/group). *$P<0.05$, **$P<0.01$ vs. saline group as determined by ANOVA followed by Dunnett's test.

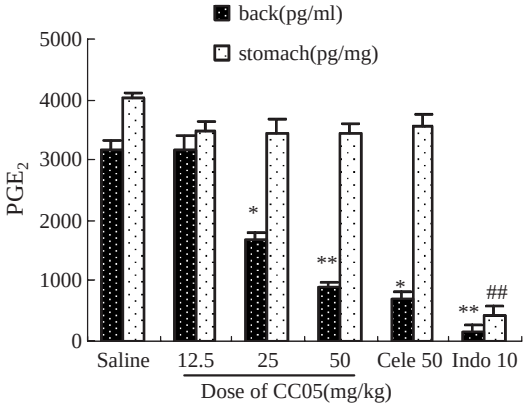

Fig. 3. Inhibition of PGE_2 production in the inflamed rat air pouch and stomach mucosa by CC05. CC05 was administered intragastrically12 h before initiating an inflammatory response in the air pouch with carrageenan. Celecoxib (50 mg/kg, i.g.) and indometacin (10 mg/kg, i.g.) were administered 0.5 h before carrageenan injection. PGE_2 present in the pouch exudates and extracted from the stomach mucosa was quantified by RIA. Each column presents as means±S.E.M. ($n=6-8$/group). *$P<0.05$, **$P<0.01$, ##$P<0.01$ vs. saline group as determined by ANOVA followed by Dunnett's test.

Table 2
Effect of CC05 and reference compounds (celecoxib and indomethacin) on cyclooxygenase-1 and cyclooxygenase-2 activities in Sf9 cells expressing human *COX-1* and *COX-2* proteins

	Cyclooxygenase-1	Cyclooxygenase-2	Ratio of
	(IC_{50})[a] (μM)	(IC_{50})[a] (μM)	*COX-2*/*COX-1*
CC05	14.34±0.05	0.328±0.04	0.0229
Celecoxib	2.4±0.01	0.053±0.01	0.0221
Indomethacin	0.00615±0.0003	0.0917±0.001	14.91

[a] Triplicate experiments were run in parallel for both *COX-1* and *COX-2*, with five concentrations from 0.1 to 1000 μM. GraphPad Prism 4.0 software was used for calculating the IC_{50} values.

3.4. Effects of CC05 on COX-2 mRNA and protein expression

Differentiated THP-1 cells serve as an excellent model system for identification of *COX-2* mRNA and protein expression (Barrios-Rodiles et al., 1996; Dreskin et al., 2001). As shown in Fig. 4A, RNA extracted from macrophages subjected to reverse transcription and real time PCR analysis indicated that LPS markedly induced mRNA expression of *COX-2* by approximately 6-fold ($n=4$, $P<0.05$ on two-way repeated measures ANOVA). Pretreatment with CC05 (5, 20 μM), indometacin (20 μM) and celecoxib (10 μM) did not prevent the induction of *COX-2* mRNA: *COX-2* mRNA expression was still increased by 6, 7-fold ($n=5$, $P<0.05$ versus control). Conversely, pretreatment with dexamethasone (10 μM) suppressed the expression of *COX-2* mRNA: *COX-2* mRNA expression was only 1.5-fold ($n=5$, $P<0.05$ versus LPS).

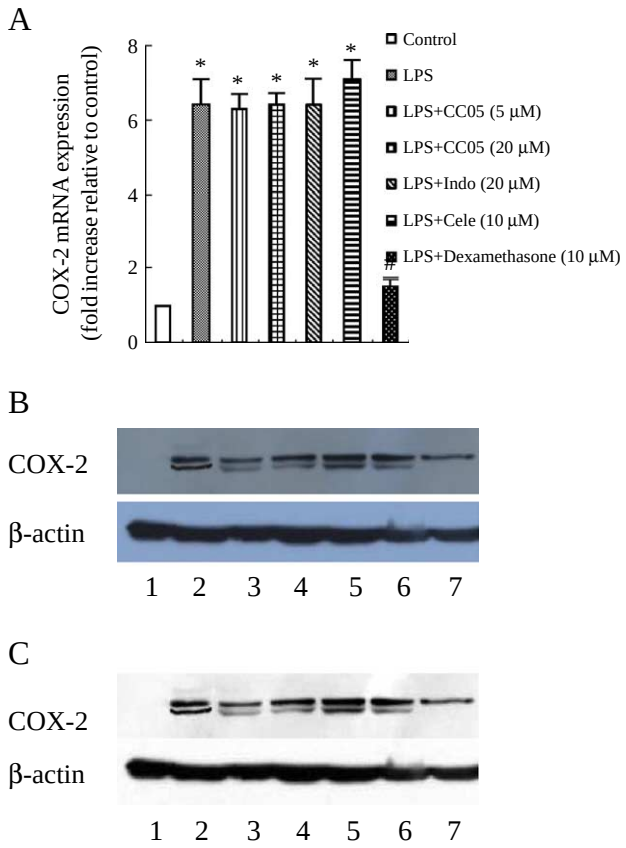

Fig. 4. LPS induces *COX-2* mRNA (real time PCR) and protein (Western blot) expression in PMA differentiated THP-1 cells. (A) This *COX-2* mRNA induction is not blocked by CC05, indomethacin and celecoxib. Conversely, *COX-2* mRNA expression is suppressed by dexamethasone. **P*, #*P*<0.05 versus control as determined by two-way repeated measures ANOVA. (B) Induction of *COX-2* protein expression is blocked by dexamethasone, but not blocked by CC05, indomethacin and celecoxib. Lane 1, control; Lane 2, 4 h incubation with LPS; Lane 3 to 9, incubation with tested drugs 30 min prior to LPS induction: Lane 3, CC05 (5 μM); Lane 4, CC05 (20 μM); Lane 5, indomethacin (20 μM); Lane 6, celecoxib (10 μM); Lane 7, dexamethasone (10 μM).

Corresponding results were obtained on Western blot analysis. A prominent band at 72 kDa was observed in LPS treated macrophages, indicating *COX-2* protein expression in these samples. No *COX-2* protein was evident in control macrophages. However, pretreatment with CC05, indomethacin and celecoxib did not affect LPS-induced *COX-2* protein expression (Barrios-Rodiles et al., 1996), in contrast to dexamethasone, which reduced the *COX-2* band. A representative immunoblot of *COX-2* protein expression in macrophages is shown in Fig. 4B.

4. Discussion

In the present study, we presented evidence that the benzenesulfonamide derivatives CC05, inhibits *COX-2* enzymatic activity in vitro and in vivo. CC05 possessed moderate potential and selective inhibition of *COX-2* enzymatic activity with an IC_{50} value of 0.328 ±0.04 μM in cell-based assay (Table 2). Our study also demonstrated that CC05 was a moderate potential and selective inhibitor of *COX-2* in two in vivo assays (Figs. 2 and 3). The most important result was that CC05 had more potential effect on paw edema reduction (50 mg/kg, i.g. inhibition 71.33%) than that of celecoxib (50 mg/kg, i.g. inhibition 44.89%). Our results were consist with Francischi et al. (2002) had reported that celecoxib only reduce edema at higher dose. The mechanism underlied *COX-2* selective inhibitor with little reduction of edema was still unclear. In our study, CC05 was administered intragastrically 12 h before carrageenan challenging since the results of HPLC-electrospray mass spectrometry (HPLC-MS) showed that plasma drug concentration reached peak at that point (data not shown). And CC05 could exert its maximal anti-inflammatory effect at that time point. Given 0.5 h before carrageenan challenging, celecoxib and indomethacin deserved mark anti-inflammation (Francischi et al., 2002). In order to compare the maximal potencies of these drugs, we choose different administration protocols.

Whittle et al. (1980) first reported that some NSAIDs can selectively inhibit PGs biosynthesis in different tissues in vivo. Injection of a carrageenan solution into the pouch produces an inflammatory reaction that is characterized by an infiltration of cells, an increase in exudates, and a marked production of biochemical mediators such as prostaglandins, leukotrienes, and cytokines. Futaki et al. (1993) reported the selective inhibition of NS-398 on PGs production in inflamed tissue, such as carrageenan induced air pouch, but not the normal gastric mucosa in rats. Thus, the carrageenan induced air pouch model has been extensively used to analyze the selectively potential of inhibition on *COX-1*/*-2* in vivo. This model is also an acute or sub-acute model to mimic clinical arthritis or other chronic inflammatory disorders since the pouch consists mainly of macrophages and fibroblasts and bears a remarkable resemblance to a synovial cavity (Futaki et al., 1993).

CC05 could inhibit PGE_2 production in the air pouch rather than PGE_2 in the stomach (Fig. 3). The result suggested that CC05 had selective action on *COX-2* in vivo. We also observed that CC05 did not produce any gastric or intestinal lesions after a single dose of 1.25 g/kg administration intragastrically in a 2-week study in mice (data not shown). These findings suggest that CC05 might be a promising candidate agent in the management of chronic rheumatoid arthritis and osteoarthritis with less usage of concomitant gastroprotective drugs.

The human monocytic leukemia cell line, THP-1, can be induced to differentiate into macrophage-like cells by treatment with 10 nM PMA for 48 h (Schwende et al., 1996). The differentiated cells adhere, stop proliferating and increase expression of the receptor of LPS, CD14. In addition, their response to LPS is greatly enhanced. Thus, we choose this model for study of the effect of CC05 on *COX-2* gene expression. At the same time, we also examined the effect of one nonspecific *COX-2* inhibitor (indomethacin), a *COX-2* specific inhibitor (celecoxib), and a glucocorticoid (dexamethasone) on the level of transcription and translation of *COX-2*. Our results indicated that both the mRNA and the protein expression of *COX-2* were suppressed by dexamethasone. Indomethacin and celecoxib did not inhibit the expression of *COX-2* gene. These results were consistent with that of Barrios-Rodiles et al. (1996). Neither the mRNA nor the protein expression level was affected by CC05 (Fig. 4). These results demonstrated that the anti-inflammatory action of CC05 is not directed towards the transcription or translation of the *COX-2* gene but only to the enzymatic activity of the protein.

However, the *COX-1*/*-2* model did not appear to explain everything. Even though in inflammatory models *COX-2* inhibitors were as active as traditional NSAIDs (Vane, 2000), there still existed some confusing issues. For example, *COX-2* inhibitors did not decrease the use of concomitant gastroprotective treatment when they were used in the treatment of osteoarthritis (Bouee et al., 2004). It is reported that kidney, lung, and brain all constitutive express *COX-2* (Guan et al., 1997; Kaufmann et al., 1996). The *COX-2* also had physiological roles, for instance, in the maintenance of fluid balance by the kidney (Khan et al., 2002); regulating the behavioral response to IL-1 (Swiergiel and Dunn, 2002); enhancing the protective effect of hepatocyte growth factor (Warzecha et al., 2004). CC05 was shown to inhibit *COX-2* activity selectively with moderate potency in our study. And CC05 had more effect on paw edema reduction than *COX-2* selective inhibitor celecoxib at the same dose. CC05 might be a novel anti-inflammatory agent, which can keep the balance of *COX-1*/-2 model and alleviate *COX-2* physiologic features related side effects.

In conclusion, the data presented here demonstrated that a novel *COX-2* inhibitor, CC05, exerted anti-inflammatory effects in vivo and in vitro. This effect did not direct towards the transcription or translation of the *COX-2* gene but mainly to the enzymatic activity of the protein.

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References

Barrios-Rodiles, M., Keller, K., Belley, A., Chadee, K., 1996. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase-2 enzyme activity but not mRNA expression in human macrophages. Biochem. Biophys. Res. Commun. 225, 896–900.

Bouee, S., Charlemagne, A., Fagnani, F., Le Jeunne, P., Sermet, C., Naudin, F., Lancry, P.J., 2004. Changes in osteoarthritis management by general practitioners in the *COX-2* inhibitor era-concomitant gastroprotective therapy. Jt. Bone Spine 71, 214–220.

DeWitt, D.L., Smith, W.L., 1988. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc. Natl. Acad. Sci. U. S. A. 85, 1412–1416.

Dreskin, S.C., Thomas, G.W., Dale, S.N., Heasley, L.E., 2001. Isoforms of Jun kinase are differentially expressed and activated in human monocyte/macrophage (THP-1) cells. J. Immunol. 166, 5646–5653.

Francischi, J.N., Chaves, C.T., Moura, A.C., Lima, A.S., Rocha, O.A., Ferreira-Alves, D.L., Bakhle, Y.S., 2002. Selective inhibitors of cyclooxygenase-2 (*COX-2*) induce hypoalgesia in a rat paw model of inflammation. Br. J. Pharmacol. 137, 837–844.

Futaki, N., Arai, I., Hamasaka, Y., Takahashi, S., Higuchi, S., Otomo, S., 1993. Selective inhibition of NS-398 on prostanoid production in inflamed tissue in rat carrageenan-air-pouch inflammation. J. Pharm. Pharmacol. 45, 753–755.

Guan, Y., Chang, M., Cho, W., Zhang, Y., Redha, R., Davis, L., Chang, S., Dubois, R.N., Hao, C.M., Breyer, M.D., 1997. Cloning, expression, and regulation of rabbit cyclooxygenase-2 in renal medullary interstitial cells. Am. J. Physiol., Renal Fluid Electrolyte Physiol. 273, 18–26.

Harris, R.C., McKanna, J.A., Akai, Y., Jacobson, H.R., Dubois, R.N., Breyer, M.D., 1994. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J. Clin. Invest. 94, 2504–2510.

Hashimoto, H., Imamura, K., Haruta, J., Wakitani, K., 2002. 4-(4-cycloalkyl/aryl-oxazol-5-yl)benzenesulfonamides as selective cyclooxygenase-2 inhibitors: enhancement of the selectivity by introduction of a fluorine atom and identification of a potent, highly selective, and orally active *COX-2* inhibitor JTE-522(1). J. Med. Chem. 45, 1511–1517.

Hawkey, C.J., 1999. *COX-2* inhibitors. Lancet 353, 307–314.

Heid, C.A., Stevens, J., Livak, K.J., Williams, P.M., 1996. Real time quantitative PCR. Genome Res. 6, 986–994.

Herschman, H.R., 1996. Prostaglandin synthase 2. Biochim. Biophys. Acta 1299, 125–140.

Hla, T., Maciag, T., 1991. Cyclooxyenase gene expression is down-regulated by heparin-binding (acidic fibroblast) growth factor-1 in human endothelial cells. J. Biol. Chem. 266, 24059–24063.

Hla, T., Neilson, K., 1992. Human cyclooxygeanse-2 cDNA. Proc. Natl. Acad. Sci. U. S. A. 89, 7384–7388.

Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.

Kaufmann, W.E., Worley, P.F., Pegg, J., Bremer, M., Isakson, P., 1996. *COX-2*, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. Proc. Natl. Acad. Sci. U. S. A. 93, 2317–2321.

Khan, K.N., Paulson, S.K., Verburg, K.M., Lefkowith, J.B., Maziasz, T.J., 2002. Pharmacology of cyclooxygenase-2 inhibition in the kidney. Kidney Int. 61, 1210–1219.

Kujubu, D.A., Fletcher, B.S., Varnum, B.C., Lim, R.W., Herschman, H.R., 1991. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J. Biol. Chem. 266, 12866–12872.

Masferrer, J.L., Zweifel, B.S., Manning, P.T., Hauser, S.D., Leahy, K.M., Smith, W.G., Isakson, P.C., Seibert, K., 1994. Selective inhibition of inducible cyclooxygenase-2 in vivo is anti-inflammatory and non-ulcerogenic. Proc. Natl. Acad. Sci. U. S. A. 91, 3228–3232.

Mitchell, J.A., Warner, T.D., 1999. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. Br. J. Pharmacol. 128, 1121–1132.

Moroney, M.A., Alcaraz, M.J., Forder, R.A., Carey, F., Hoult, J.R., 1988. Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. J. Pharm. Pharmacol. 40, 787–792.

Penning, T.D., Talley, J.J., Bertenshaw, S.R., Carter, J.S., Collins, P.W., Docter, S., Graneto, M.J., Lee, L.F., Malecha, J.W., Miyashiro, J.M., Rogers, R.S., Rogier, D.J., Yu, S.S., Anderson, G.D., Burton, E.G., Cogburn, J.N., Gregory, S.A., Koboldt, C.M., Perkins, W.E., Seibert, K., Veenhuizen, A.W., Zhang, Y.Y., Isakson, P.C., 1997. Synthesis and biological evaluation of the 1, 5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). J. Med. Chem. 40, 1347–1365.

Prasit, P., Wang, Z., Brideau, C., Chan, C.C., Charleson, S., Cromlish, W., Ethier, D., Evans, J.F., Ford-Hutchinson, A.W., Gauthier, J.Y., Gordon, R., Guay, J., Gresser, M., Kargman, S., Kennedy, B., Leblanc, Y., Léger, S., Mancini, J., O'Neill, G.P., Ouellet, M., Percival, M.D., Perrier, H., Riendeau, D., Rodger, I., Tagari, P., Thérien, M., Vickers, P., Wong, E., Xu, L.J., Young, R.N., Zamboni, R., 1999. The discovery of rofecoxib, [MK 966, VIOXX®, 4-(4′-methylsulfonylphenyl)-3-phenyl-2(*5H*)-furanone], an orally active cyclooxygenase-2 inhibitor. Bioorg. Med. Chem. Lett. 9, 1773–1778.

Schwende, H., Fitzke, E., Ambs, P., Dieter, P., 1996. Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. J. Leukoc. Biol. 59, 555–561.

Sedgwick, A.D., Lees, P., 1986. Studies of eicosanoid production in the air pouch model of synovial inflammation. Agents Actions 18, 429–438.

Sedgwick, A.D., Sin, Y.M., Edwards, J.C., Willoughby, D.A., 1983. Increased inflammatory reactivity in newly formed lining tissue. J. Pathol. 141, 483–495.

Smith, W.L., Wilkin, G.P., 1977. Immunochemistry of prostaglandin endoperoxide-forming cyclooxygenases: the detection of the cyclooxygenases in rat, rabbit and guinea pig kidneys by immnofluorescence. Prostaglandins 13, 873–892.

Swiergiel, A.H., Dunn, A.J., 2002. Distinct roles for cyclooxygenases 1 and 2 in interleukin-1-induced behavioral changes. J. Pharmacol. Exp. Ther. 302, 1031–1036.

Vane, J.R., 2000. The fight against rheumatism: from willow bark to *COX-1* sparing drugs. J. Physiol. Pharmacol. 51, 573–586.

Vane, J.R., Botting, R.M., 1998. Mechanism of action of anti-inflammatory drugs. Int. J. Tissue React. 20, 3–15.

Warzecha, Z., Dembinski, A., Ceranowicz, P., Konturek, S., Tomaszewska, R., Stachura, J., Nakamura, T., Konturek, P.C., 2004. Inhibition of cyclooxygenase-2 reduces the protective effect of hepatocyte growth factor in experimental pancreatitis. Eur. J. Pharmacol. 486, 107–119.

Whittle, B.J., Higgs, G.A., Eakins, K.E., Moncada, S., Vane, J.R., 1980. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. Nature 284, 271–273.

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

Wright, J.M., 2002. The double-edged sword of *COX-2* selective NSAIDs. CMAJ 167, 1131–1137.

Xie, W.L., Chipman, J.G., Robertson, D.L., Erikson, R.L., Simmons, D.L., 1991. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Natl. Acad. Sci. U. S. A. 88, 2692–2696.

Zhang, W.Y., Yang, X.N., Jin, D.Z., Zhu, X.Z., 2004. Expression and enzyme activity determination of human cyclooxygenase-1 and-2 in a baculovirus-insect cell system. Acta. Pharmacol. Sin. 25, 1000–1006.