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SELECTIVE INHIBITION OF CYCLOOXYGENASE 2

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Abstract—Cyclooxygenase (COX), a key enzyme in the formation of prostanoids, is known to exist in two isoforms: an inducible enzyme (COX 2) and a constitutive form (COX 1). Both enzymes are inhibited by non-steroidal anti-inflammatory drugs (NSAID), but only marginal selectivity has thus far been reported. In this study, we report on a novel selective inhibitor of COX 2, CGP 28238 (6-(2,4-difluorophenoxy)-5-methyl-sulfonylamino-1-indanone). Human washed platelets were used as a source of COX 1. For IL-1 stimulated rat mesangial cells we demonstrated the almost exclusive presence of COX 2 in western blot and mRNA analysis. Therefore these two model systems were chosen for selectivity testing. With an IC_{50} value of 15 nM, CGP 28238 blocked COX 2 activity in a similar concentration range to that of other potent NSAID such as indomethacin and diclofenac (IC_{50} = 1.17–8.9 nM). However, in contrast to these reference NSAIDs, CGP 28238 was at least 1000-fold less potent in inhibiting COX 1. Using other cell systems reported to express COX 1 or COX 2, we obtained a similar selectivity for COX 2. Thus, on the basis of our findings, CGP 28238 is a novel, highly potent and selective inhibitor of COX 2 and may be a lead compound for a new generation of potent anti-inflammatory drugs with an improved side-effect profile.

Key words: non-steroidal anti-inflammatory drugs; cyclooxygenase; arachidonic acid metabolism; interleukin 1; mesangial cell; reverse transcription-polymerase chain reaction

The prostanoids, consisting of prostaglandins, prostacyclin and thromboxane, act locally in a paracrine and autocrine manner and modulate cell and tissue responses in physiological and pathophysiological processes. Recent findings have demonstrated that two isoenzymes of COX§ catalyse the conversion of AA to PGH_2, the precursor for prostanoid biosynthesis. Because of this central function in the arachidonic acid cascade, the enzyme is of physiological and pharmacological importance in the regulation of prostanoid synthesis.

The isoforms COX 1 and COX 2 are encoded by separate genes and differ in their regulation and tissue distribution. COX 1, a housekeeping gene expressed constitutively, is assumed to be responsible for producing prostanoids for physiological functions such as vascular homeostasis, regulation of renal blood flow and maintenance of glomerular filtration rate [1]. COX 2, an immediate early gene during inflammatory responses, appears to be expressed only by specific stimulatory events. Inflammation mediators such as growth factors, cytokines and endotoxin dramatically induce the enzyme expression in different cellular systems [2, 3]. Furthermore, the induction of this isoform is downregulated by glucocorticoids [4]. This has led to the hypothesis that a highly expressed COX 2 is responsible for the high levels of prostanoids present in inflamed tissues. It is generally accepted that both cyclooxygenases are important targets for NSAID [5, 6]. Aspirin, indomethacin and ibuprofen inhibit COX 1 and COX 2, which explains their anti-inflammatory effects as well as their most common side-effects, gastro-intestinal bleeding and renal dysfunction [7].

Although COX 1 and COX 2 proteins share a high degree of homology (60–70%), several studies with available NSAID have demonstrated that the two isozymes can be inhibited differentially [8, 9] but a factor of 8 for preferential COX 2 inhibition over COX 1 has been the maximum reported [5]. We investigated the inhibitory action of a novel compound, CGP 28238, for which potent anti-inflammatory activity and excellent gastro-intestinal tolerability has been reported [10, 11]. Our data point to a minimum of more than 1000-fold higher inhibitory activity of CGP 28238 for COX 2 over COX 1 for this novel NSAID.

MATERIALS AND METHODS

Materials

Cell culture media were obtained from GIBCO (Eggenstein, F.R.G.) except for the insulin-transferrin-selenite supplement (Boehringer Mannheim, Mannheim, F.R.G.). Recombinant human (rh)IL-1β was kindly provided by Dr K. Vosbeck (Ciba, Basel, Switzerland). [^{14}C]-Arachidonic acid (53 mCi/mmol) was purchased from DuPont (Dreieich, F.R.G.); organic solvents were obtained from Merck (Darmstadt, F.R.G.);

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§ Abbreviations: COX, cyclooxygenase; IL-1, interleukin 1β; NSAID, non-steroidal anti-inflammatory drugs; RMC, rat mesangial cells; AA, arachidonic acid; TxA_2, thromboxane A_2; PGH_2, 15(*S*)-hydroxy-9,11-[epidioxy]-prosta-5,13(Z,E)-dienoic acid; RT-PCR, reverse transcription-polymerase chain reaction.

and polyclonal antibodies directed against COX 2 as well as prostanoid standards were from Cayman Chemicals (Gif, France). The ECL kit and nitrocellulose membranes (Hybond-C) were purchased from Amersham (Braunschweig, F.R.G.). Diclofenac as well as indomethacin were purchased from Sigma (Deisenhofen, F.R.G.). Primers were synthesized by MWG-Biotech (Ebersberg, F.R.G.), AmpliTaq polymerase was obtained from Perkin-Elmer (Weiterstadt, F.R.G.) and Superscript reverse transcriptase from GIBCO (Eggenstein, F.R.G.). CGP 28238 was kindly provided by Dr I. Wiesenberg-Boettcher (Ciba, Basel, Switzerland).

Cell culture

Human washed platelets and monocytes were isolated from fresh blood as reported recently [12, 13].

Rat mesangial cells were used between passage 5 and 10 (with a constant split ratio of 1:3) [14]. Cultures were maintained under growth conditions in RPMI 1640 supplemented with insulin (5 μg/mL), transferrin (5 μg/mL), sodium selenite (5 μg/mL), L-glutamine (1%), penicillin (100 U/mL), streptomycin (100 μg/mL), containing 10% FCS; growth was then arrested by low serum conditions (0.5% FCS) without penicillin and streptomycin for 24 hr. The cells were stimulated under low serum conditions with IL-1β (1 nM) for 24 hr and the supernatants collected for determination of endogenously generated 6-keto-$PGF_{1\alpha}$ by radioimmunoassay [15] and nitrite by the Griess reagent [16]. Cells were washed and fresh medium was added 15 min prior to arachidonic acid stimulation.

Differential western blot analysis

Following 24 hr stimulation mesangial cells were washed twice with ice-cold PBS scraped off in PBS containing 15 mM EDTA, 1 mM PMSF, 10 μg/mL aprotinin and 0.2 mg/mL α_2-macroglobulin, and pelleted. Cell pellets were solubilized in the same buffer containing 1% Triton X-100 (lysis buffer).

Immunoblot analysis for both COX isoforms was performed as follows: solubilized cell lysates with equal amounts of protein were separated by a 10% SDS–PAGE and blotted for 1.5 hr with a constant current of 300 mA on to a nitrocellulose membrane in a semidry blot procedure (25 mM Tris/192 mM glycine/20% methanol). Proteins were visualized with a 5% solution of Ponceau S to check transfer efficiency. After destaining, the membrane was blocked with 5% milk powder in PBS/0.1% Tween 20 for 2 hr. A polyclonal antibody directed against COX 2 was applied in a dilution of 1:500 for 2 hr in a humid chamber. The blot was washed several times with PBS–Tween and incubated with a goat anti-rabbit antibody in a dilution of 1:7500 for 45 min. For detection of COX 1, the membrane [after stripping off anti-COX 2 antibody with 100 mM Tris–HCl/2% SDS/100 mM β-mercaptoethanol (pH 6.7) for 30 min at 60°] was reprobed with a polyclonal antibody [13] which cross-reacts with both isoforms. Antibody binding was visualized by the enhanced chemiluminescence technique, according to the instructions of the supplier (Amersham).

Cyclooxygenase activity and product characterization in whole cells and cell lysates

Whole cells. After preincubation with different stimuli, cells were rinsed twice with PBS and the different cyclooxygenase inhibitors added. Following 15 min incubation the cells were washed and stimulated with ^{14}C-labelled arachidonic acid (final concentration 2 μM) or 5 μM [^{14}C]PGH_2 synthesized as described in [17], for 15 min and 5 min, respectively, at 37°. The reaction was stopped by scraping off the cells with a rubber policeman and the cell suspension was transferred to 4 v/v ethylacetate. Radioactive products were extracted at pH 3 into the organic solvent, gently evaporated under N_2 and subsequently separated by thin layer chromatography (ethyl-acetate:H_2O:isooctane:acetic acid/90:100:50:20). The amount of prostanoid production was quantified by autoradiography, following visualization with a phosphoimaging system (Image Quant; Molecular Dynamics) and expressed as % radioactivity of total amount of extracted radioactivity. Thereafter, the location of authentic prostanoids was visualized with iodine vapour, and the radioactive zones were identified. Prostacyclin synthesis was monitored by the formation of 6-keto-$PGF_{1\alpha}$, and TxA_2 formation by its stable hydrolysis product TxB_2. IC_{50} values were determined by computeral analysis (Graphpad) from at least four independent experiments.

Cell homogenates. Stimulated RMC or platelets were sonificated in lysis buffer, preincubated for 15 min with the indicated inhibitors and then stimulated in the presence of the cofactors hematine (2 μM), glutathione (5 mM) and L-tryptophan (5 mM) with 10 μM [^{14}C]AA for 15 min at 37°. The following steps were performed as described above.

Whole cell RT-PCR analysis

Cells (1.2×10^6) were washed in PBS, and resuspended in DEPC-treated H_2O containing 10 U RNasin and lysed for 30 min on ice. Cells were pelleted by centrifugation and the RNA-containing supernatant was used for specific reverse transcription with Superscript reverse transcriptase and the wobble primer PCOXR1(5′-A(G/C)AGCTCAGT(G/T)-GA(A/G)CG(C/T)CT-3′) complementary to a homologous 3′-part of mouse COX 1 and 2. This approach allowed a specific and simultaneous transcription of both COX-isoenzymes. After removal of excess primers for transcription, PCR amplification was performed using the cDNA with the primer PCOXF1 (5′-GGAGAGAAGGA(A/G)ATGGCTGC-3′), corresponding to a homologous 5′-part of COX 1 and 2, and PCOXR2 (5′-ACCCGTCATCTCCAGGGTAA-3′) as antisense primer specific for COX 1 and PCOXR3 (5′-ATCTAGTCTGGAGTGGGAGG-3′) as antisense primer specific for COX 2. The reactions were cycled 35 times in a cycle profile for 45 sec at 94°, 45 sec at 50° and 45 sec at 72° after a 5 min denaturing step at 95°. Amplification products were analysed by 2% agarose gel electrophoresis and ethidium bromide staining. The identity of the fragments was evaluated by their molecular mass and restriction enzyme pattern.

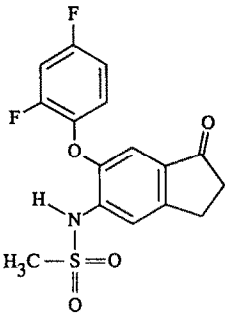

Fig. 1. Chemical structure of CGP 28238.

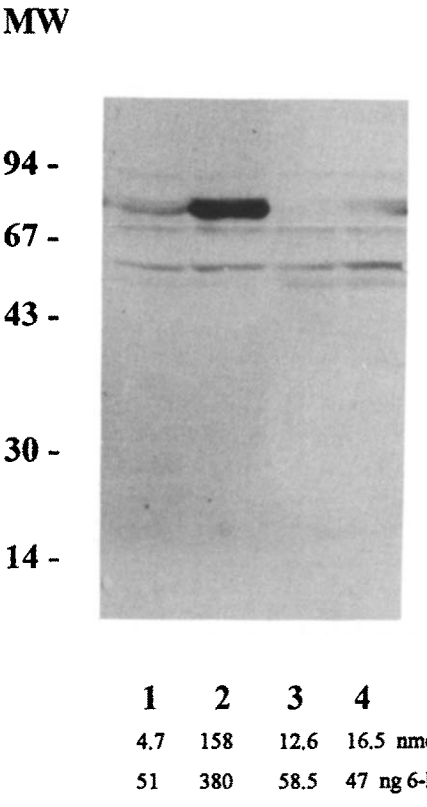

Fig. 2. Immunoblot analysis of COX 2 induction in rat mesangial cells. Detection of IL-1 induced COX 2 in rat mesangial cells with an anti-COX 2 antibody. Lane 1, control cells; lane 2, IL-1 treated RMC; lane 3, cells treated with IL-1 in the presence of 1 μM dexamethasone; lane 4, cells treated with IL-1 in the presence of 1 μM cycloheximide. Total cellular protein was 200 μg of Triton solubilized cell lysates. Values displayed below represent the endogenous production of nitrite and 6-keto-$PGF_{1\alpha}$.

RESULTS AND DISCUSSION

CGP 28238 [6-(2,4-difluorophenoxy)-5-methylsulfonylamino-1-indanone; Fig. 1] was reported to be a highly potent anti-inflammatory compound with significantly improved gastrointestinal safety. In various acute and subchronic rat models for anti-inflammatory, analgesic and antipyretic activity the compound was shown to be equipotent to indomethacin [10, 11].

To determine whether this drug, compared to reference NSAID such as indomethacin and diclofenac, exhibits a selectivity for one of the COX-isoenzymes, we used two model systems: human washed platelets, known from various studies to host only COX 1, were taken to study inhibitory effects on the constitutive enzyme; and IL-1-stimulated rat mesangial cells were used as a source of the inducible enzyme.

Immunoblot analysis with a polyclonal antibody reacting exclusively with COX 2 demonstrated a strong band of approximately 70 kD in IL-1-stimulated mesangial cells, consistent with data recently published [18]. Interestingly, a weak band for COX 2 was also observed in control cells, COX 1 being only barely detectable (data not shown). The induced appearance of the 70 kD protein was totally abolished by coincubation with 1 μM dexamethasone (Fig. 2), a typical feature of COX 2. Further, RT-PCR with primers specific for COX 1 and 2 amplified fragments of predicted sizes in control cells. In IL-1-treated cells, a dramatic elevation in the amplification of the COX 2 fragment was observed, indicating an increase in COX 2 mRNA expression. COX 1 mRNA remained unchanged (Fig. 3). As a control, amplification of a fragment of β-actin revealed no difference in IL-1-stimulated and control cells (Fig. 3). Taken together, the above data confirm our presumption of IL-1-stimulated rat mesangial cells as a suitable COX 2 model system. As an additional marker of IL-1-induced cell stimulation, the concentration of nitrite and 6-keto $PGF_{1\alpha}$ was measured in the supernatant, as it has been reported that IL-1 induces nitric oxide synthase in rat mesangial cells [19].

First, we determined the inhibitory action of indomethacin, diclofenac and CGP 28238 on COX 1 in human platelets. As shown in Table 1 and Fig. 4A, all compounds inhibited the conversion of exogenously added arachidonic acid to TxB_2 concentration dependently, although with remarkably different potencies. Whereas indomethacin and diclofenac inhibited with IC_{50} values of 1.5 nM and 17.9 nM, respectively, an inhibitory action of CGP 28238 could only be observed at much higher concentrations (Table 1). The determined IC_{50} of 72.3 μM for the inhibition of COX 1 is about 50,000-fold higher than the IC_{50} value of indomethacin and 4000-fold higher than the IC_{50} of diclofenac.

Next, we analysed the inhibitory potential of these NSAIDs on intact rat mesangial cells (COX 2). For CGP 28238 we determined an IC_{50} value of 14.7 nM, showing an equipotent inhibition of COX 2 compared to indomethacin (IC_{50} = 8.9 nM) and diclofenac (IC_{50} = 1.2 nM). This is of interest since CGP 28238, indomethacin and diclofenac are comparably active as anti-inflammatory compounds. The ratio $IC_{50\text{-}COX\,1}/IC_{50\text{-}COX\,2}$ of approximately 5000 for CGP 28238 demonstrates a high selectivity of

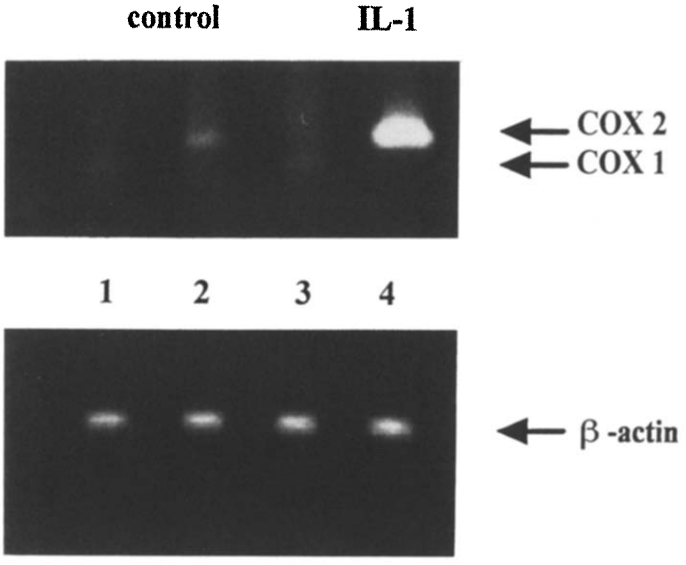

Fig. 3. Whole cell PCR of rat mesangial cells for COX 1 and COX 2. RT-PCR analysis of the cyclooxygenase isoform mRNA of 1.2×10^6 of non-stimulated or IL-1-stimulated cells was performed as described under Materials and Methods. Lanes 1 and 2, control cells; lanes 3 and 4, IL-1 stimulated cells; the primers used were PCOX F1 and PCOX R2 for COX 1 (lanes 1 and 3) and PCOX F1 and PCOX R3 for COX 2 (lanes 2 and 4). The control amplified mRNA for β-actin is indicated below.

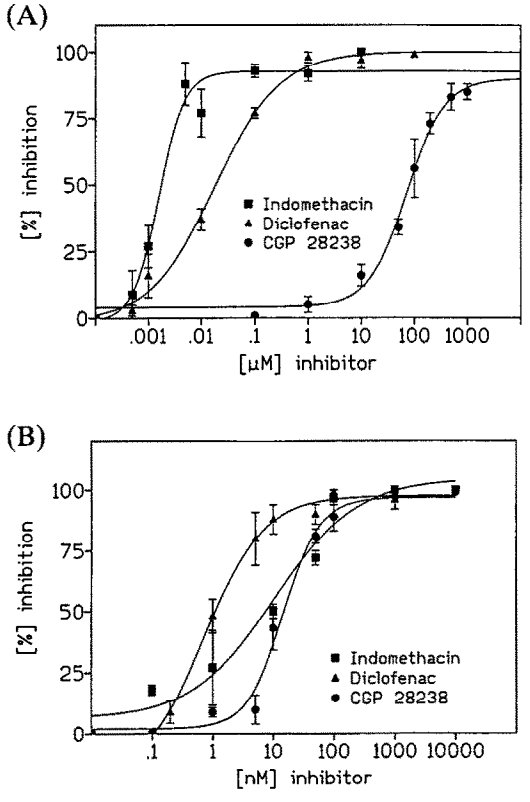

Fig. 4. Inhibitory effect of different NSAID on platelets COX 1 (A) and induced rat mesangial cell COX 2 (B). Cells were preincubated with the indicated concentrations of the different NSAID for 15 min and 5 μM [^{14}C]AA was then added. Generated prostanoids were chromatographed and IC_{50} values determined as described under Materials and Methods. All values represent four independent experiments ± SE. The IC_{50} values determined are summarized in Table 1.

Table 1. IC_{50} values of different NSAID in platelets and in mesangial cells

	CGP 28238	Indomethacin	Diclofenac
Platelets (COX 1)	72.3 μM	1.5 nM	17.9 nM
RMC (COX 2)	14.7 nM	8.9 nM	1.2 nM
Ratio	~5000	0.15	15

The ratio ($IC_{50\text{-COX }1}/IC_{50\text{-COX }2}$) gives a description of efficiency in inhibition of COX 2 relative to COX 1. The IC_{50} values were determined from the concentration dependencies in Fig. 4.

Table 2. Effect of CGP 28238 inhibition on thromboxane and prostacyclin synthase activity

	Control	CGP 28238
Platelets TxB_2	32.3 ± 0.66	32.4 ± 1.6
RMC 6-keto-$PGF_{1\alpha}$	1.9 ± 0.2	2.1 ± 0.4

Platelets or IL-1-stimulated RMC were incubated for 5 min with 5 μM [^{14}C]PGH_2 in the presence or absence of 500 μM CGP 28238. The given products were analysed by thin layer chromatography. The values represent % conversion of total extracted radioactivity.

this drug for COX 2. These results may explain the favourable side-effect profile of CGP 28238 in comparison to indomethacin and diclofenac [11].

Comparing our results with published data, the selectivity of CGP 28238 for COX 2 is about 700-fold higher than that of 6-methoxy-2-naphthyl acetic acid [5], the active metabolite of nabumetone, and

Table 3. IC_{50} values for CGP 28238 inhibition on the metabolism of endogenously released arachidonic acid

	CGP 28238	Stimulus
Platelets (COX 1)	42 μM	Thrombin [1 U/mL]
RMC (COX 2)	35 nM	Angiotensin II [100 nM]

Human platelets were labelled with [^{14}C]AA for 2 hr. After washing, the platelets were stimulated for 3 min with thrombin (1 U/mL) and TxB_2 formation was identified by thin layer chromatography. The conversion of endogenous AA to 6-keto $PGF_{1\alpha}$ in IL-1 stimulated RMC, treated with 100 nM angiotensin II for 30 min, was measured in cell supernatants by RIA. IC_{50} values were calculated from inhibition of increasing concentrations of CGP 28238.

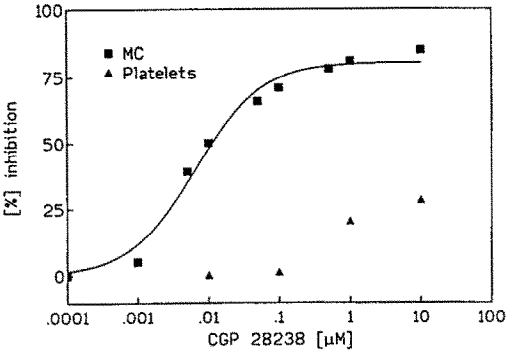

Fig. 5. Concentration dependency of CGP 28238 inhibition in cell homogenates of RMC and platelets. Platelets or IL-1-stimulated RMC were disrupted in the presence of 1% Triton as described and incubated with 5 μM [^{14}C]AA in the presence of the indicated concentration of CGP 28238. After extraction, TxB_2 and 6-keto $PGF_{1\alpha}$ formation was detected by thin layer chromatography.

1000 times greater than that of BF-389 [6]. Both compounds were reported to be more selective inhibitors of the COX 2 isoenzyme. To exclude the possibility that the inhibitory action of CGP 28238 is due to the inhibition of the secondary enzymes of the arachidonic acid metabolism, namely thromboxane synthase and prostacyclin synthase, we investigated the effect of CGP 28238 on the enzyme-catalysed conversion of PGH_2 to thromboxane and prostacyclin. Even in concentrations up to 500 μM no inhibitory effect of CGP 28238 on thromboxane synthase activity in platelets or prostacyclin synthase activity in rat mesangial cells was observed (Table 2).

In order to investigate drug effects on the conversion of AA from endogenous pools, we labeled platelets and IL-1-stimulated mesangial cells with [^{14}C]AA and then analysed the effect of CGP 28238. Platelets were stimulated with thrombin and rat mesangial cells with angiotensin II to mimic physiological situations. We observed a similar selectivity of CGP 28238 in inhibiting COX 2 in rat mesangial cells with an IC_{50} value of 35 nM compared to COX 1 inhibition in platelets (IC_{50} = 42 μM; Table 3). Notably, the phospholipase A_2 step was unaffected by CGP 28238 (unpublished results).

To exclude the possibility that the availability of AA across the membrane or from phospholipids was affected by CGP 28238 and consequently that an interaction upstream of the COX step might occur, we studied the inhibitory potential of the compound in cell lysates. As seen in Fig. 5, CGP 28238 inhibited solubilized COX 2 with an IC_{50} value of approximately 10 nM to the same extent as in intact mesangial cells. COX 1 in homogenates of platelets was only inhibited in concentrations above 10 μM.

To extend these findings further we also tested the inhibition of COX activity by CGP 28238 in other well-characterized cell systems (Table 4). In TPA-treated HEL cells which after 72 hr stimulation express COX 1 exclusively [20, 21] and in LPS-stimulated human monocytes, expressing high levels of COX 2 after 14 hr of stimulation, we obtained comparable results. CGP 28238 was nearly inactive in inhibiting prostanoid production in HEL-cells (COX 1: $IC_{50} > 10$ μM), but very potent in LPS-stimulated monocytes (COX 2: IC_{50} = 38 nM).

Table 4. IC_{50} values for CGP 28238 inhibition in human monocytes and HEL cells

	CGP 28238	Stimulus
HEL cells (COX 1)	>10 μM	TPA [100 nM] 70 hr
Monocytes(COX 2)	38 nM	LPS [1 μg/mL] 14 hr

HEL cells and human monocytes were treated as indicated below. The cells were preincubated for 15 min with various concentrations of CGP 28238. Five micromolar [^{14}C]AA was added for 15 min and prostanoids were extracted and determined as described under Materials and Methods.

In summary, we report here the inhibitory profile of a highly specific COX 2 inhibitor. The metabolism of endogenous and exogenous AA in cells predominantly or exclusively containing COX 2 and also by the solubilized COX 2 enzyme is blocked by CGP 28238 in nanomolar concentrations, whereas in all systems measuring COX 1 activity, CGP 28238 was only marginally active. On the basis of these results it should be possible for clinical use to synthesize selective COX 2 inhibitors which effectively suppress the production of pathologically relevant prostanoids without affecting the synthesis of prostanoids essential for physiological functions.

During preparation of this manuscript a new compound (NS-398) with a selectivity for COX 2 was reported [22]. However, no chemical structure was given, and the maximal inhibition of COX 2 isoenzyme of this compound amounted to 60%.

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REFERENCES

1. Mene P, Simonson MS and Dunn MJ, Physiology of the mesangial cell. *Physiol Rev* **69**: 1347–1423, 1989.
2. Herschman H, Fletcher B and Kujubu D, TIS10, A mitogen-inducible glucocorticoid-inhibited gene that encodes a second prostaglandin synthase/cyclooxygenase enzyme. *J Lipid Med* **6**: 89–99, 1993.
3. Meade EA, Smith WL and DeWitt DL, Expression of the murine prostaglandin (PGH) synthase-1 and PGH synthase-2 isozymes in cos-1 cells. *J Lipid Med* **6**: 119–129, 1993.
4. Simmons D, Xie W, Evett G, Merrill J, Robertson DL and Bradshaw WS, Drug inhibition and cellular regulation of prostaglandin G/H sythase isoenzyme 2. *J Lipid Med* **6**: 113–117, 1993.
5. Meade EA, Smith WL and Dewitt DL, Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other nonsteroidal antiinflammatory drugs. *J Biol Chem* **268**: 6610–6641, 1993.
6. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ and Vane JR, Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA* **90**: 11693–11697, 1993.
7. Schlondorff D, Renal complications of nonsteroidal anti-inflammatory drugs. *Kidney Int* **44**: 643–653, 1993.
8. Oneill GP and Fordhutchinson AW, Expression of mRNA for cyxlooxygenase-1 and cyclooxygenase-2 in human tissue. *FEBS Lett* **330**: 156–160, 1993.
9. DeWitt DL, Meade EA and Smith WL, PGH synthase isozyme selectivity: the potential for safer nonsteroidal antiinflammatory drugs. *Am J Med* **95**: 2A–44S, 1993.
10. Wiesenberg-Boettcher I, Schweizer A and Müller K, The pharmacological profile of CGP 28238, a highly potent anti-inflammatory compound. *Agents Actions* **26**: 240–242, 1989.
11. Wiesenberg-Boettcher I, Schweizer A, Green JA, Mueller K, Maerki F and Pfeilschifter J, The pharmacological profile of CGP 28238, a novel highly potent anti-inflammatory compound. *Drug Exp Clin Res* **XV**(11/12): 501–509, 1989.
12. Wu XB, Brüne B, von Appen F and Ullrich V, Efflux of cyclic GMP from activated human platelets. *Mol Pharmacol* **43**: 564–568, 1993.
13. Nüsing R and Ullrich V, Regulation of cyclooxygenase and thromboxane synthase in human monocytes. *Eur J Biochem* **206**: 131–136, 1992.
14. Pfeilschifter J, Kurtz A and Bauer C, Activation of phospholipase C and prostaglandin synthesis by (arginine) vasopressin in cultures of rat mesangial cells. *Biochem J* **223**: 855–859, 1984.
15. Salmon JA, A radioimmunoassay for 6-ketoPGF$_{1\alpha}$. *Prostaglandins* **15**: 383–397, 1978.
16. Matsunaga H, Oyama T and Nishimura M, Determination of nitrite and nitrate in fresh water. *Anal Chim Acta* **58**: 228–231, 1972.
17. Hecker M and Ullrich V, On the mechanism of prostacyclin and thromboxane A_2 biosynthesis. *J Biol Chem* **264**: 141–150, 1989.
18. Martin M, Neumann D, Hoff T, Resch K, DeWitt DL and Goppelt-Struebe M, Interleukin-1-induced cyclooxygenase 2 expression is supressed by cyclosporin A in rat mesangial cells. *Kidney Int* **45**: 150–158, 1994.
19. Pfeilschifter J and Schwarzenbach H, Interleukin 1 and tumor necrosis factor stimulate cGMP formation in rat mesangial cells. *FEBS Lett* **273**: 185–190, 1990.
20. Tanabe T, Yokoyama C, Miyata A, Ihara H, Kosaka T, Suzuki K, Nishikawa Y, Yoshimoto T, Yamamoto S, Nüsing R and Ullrich V, Molecular cloning and expression of human thromboxane synthase. *J Lipid Med* **6**: 139–144, 1993.
21. Ihara H, Yokoyama C, Miyata A, Kosaka T, Nüsing R, Ullrich V and Tanabe T, Induction of thromboxane synthase and prostaglandin endoperoxide mRNAs in human erythroleukemia cells by phorbol ester. *FEBS Lett* **306**: 161–164, 1992.
22. Futaki N, Takahashi M, Yokoyama I, Arai S, Higuchi S and Otomo S, NS-398, a new anti-inflammatory agent selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX 2) activity in vitro. *Prostaglandins* **47**: 55–59, 1994.