# Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes

Miyako Kato, Shinichi Nishida, Hidero Kitasato, Natsue Sakata
and Shinichi Kawai

## Abstract

Since the pharmacological profiles of various non-steroidal anti-inflammatory drugs (NSAIDs) might depend on their differing selectivity for cyclooxygenase 1 (COX-1) and 2 (COX-2), we developed a new screening method using human peripheral monocytes. Monocytes from healthy volunteers were separated, and the cells were incubated with or without lipopoly-saccharide (LPS). Monocytes without LPS stimulation exclusively expressed COX-1 on Western blotting analysis, whereas LPS stimulation induced COX-2 expression. Unstimulated monocytes (COX-1) and LPS-stimulated monocytes (COX-2) were then used to determine the COX selectivity of various NSAIDs. The respective mean IC50 values for COX-1 and COX-2 IC50 ($\mu$M), and the COX-1/COX-2 ratio of each NSAID were as follows: celecoxib, 82, 6.8, 12; diclofenac, 0.076, 0.026, 2.9; etodolac, $> 100$, 53, $> 1.9$; ibuprofen, 12, 80, 0.15; indometacin, 0.0090, 0.31, 0.029; meloxicam, 37, 6.1, 6.1; 6-MNA (the active metabolite of nabumetone), 149, 230, 0.65; NS-398, 125, 5.6, 22; piroxicam, 47, 25, 1.9; rofecoxib, $> 100$, 25, $> 4.0$; S-2474, $> 100$, 8.9, $> 11$; SC-560, 0.0048, 1.4, 0.0034. The percentage inhibition of COX-1 activity at the IC50 of COX-2 also showed a wide variation among these NSAIDs. The bioassay system using human monocytes to assess the inhibitory effects of various NSAIDs on COX-1 and COX-2 may become a clinically useful screening method.

Institute of Medical Science, St Marianna University School of Medicine, Kawasaki 216-8512, Japan

Miyako Kato, Shinichi Nishida, Hidero Kitasato, Natsue Sakata, Shinichi Kawai

Department of Microbiology, Kitasato University School of Medicine, Sagamihara 228-8555, Japan

Hidero Kitasato

Correspondence: S. Kawai, Institute of Medical Science, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8512, Japan. E-mail: s2kawai@marianna-u.ac.jp

## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) produce both their therapeutic and toxic effects by decreasing the biosynthesis of prostaglandins (PG) and other proinflammatory agents (Vane 1971). NSAIDs have been shown to decrease the production of proinflammatory PGs by inhibition of cyclooxygenase (COX) or PGG/H synthase (Flower et al 1972). Subsequent studies (Kujubu et al 1991; Xie et al 1991) have revealed that COX exists as two isozymes, which differ in their basal levels of expression, tissue localization, and inducibility during inflammation (Vane & Botting 1996). COX-1 is constitutively expressed and has been detected in every type of cell examined to date (O'Neill & Ford-Hutchinson 1993). In contrast, COX-2 expression is nearly undetectable in unstimulated cells, but this isozyme is inducible (Crofford et al 1994) and its induction coincides with an increase of inflammatory PGs in-vivo (Masferrer et al 1994).

Expression of COX-2 varies over the course of the inflammatory response (Appleton et al 1994; Anderson et al 1996). It has been proposed that inhibition of COX-1 in gastric mucosal cells and the resulting decrease of PG synthesis leads to

the loss of local protection, and that this accounts for the significant gastric toxicity of NSAIDs (Emery 1996). COX-2 activity, which leads to the production of pro-inflammatory substances, is clearly a more selective therapeutic target for NSAIDs.

The relative potency and selectivity of NSAIDs appear to correspond to their gastric toxicity, with the more COX-2-selective agents causing a lower incidence of gastric injury (Kawai 1998). Previous studies comparing the inhibition of COX-1 and COX-2 by NSAIDs have been performed using various systems, which can be categorized as enzyme systems or intact cell systems. Direct enzyme assay systems may be influenced by subtle changes of conditions, including variation in the incubation time (Laneuville et al 1994), so assay systems using living cells are more informative about the actual COX selectivity of NSAIDs (Kawai et al 1998; Warner et al 1999; Yamazaki et al 1999). However, previous studies have usually employed different kinds of cells to assess COX-1 and COX-2, or have used mixed cell cultures. The present study was designed to characterize the COX-1 and COX-2 inhibitory activities of various NSAIDs using human monocytes alone.

## Materials and Methods

### Reagents

RPMI-1640 medium, penicillin–streptomycin (PC-SM) solution, and fetal bovine serum (FBS) were obtained from Gibco BRL (Gaithersburg, MD). Ficoll-paque and Rainbow-colored protein molecular weight markers were obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). Lipopolysaccharide (LPS; *E. coli* 055: B5) was obtained from Difco Laboratories (Detroit, MI). Aspirin, diclofenac, ibuprofen, indometacin and piroxicam were purchased from Sigma Chemical Co. (St Louis, MO). Celecoxib and rofecoxib were synthesized according to Penning et al (1997) and International Patent Publication WO95/00501, respectively. Etodolac (Wyeth Lederle Japan, Tokyo, Japan), meloxicam (Nippon Boehringer Ingelheim Co., Ltd, Hyogo, Japan), 6-methoxy-2-naphthylacetic acid (the active metabolite of nabumetone: 6-MNA; SmithKline Beecham Japan, Tokyo, Japan), S-2474 (Shionogi & Co., Ltd, Osaka, Japan) and SC-560 (Searle, Skokie, IL) were obtained from the listed companies. A DC Protein Assay kit (Bio-Rad, Hercules, CA), HistoScan (Biomeda, Foster City, CA), and acrylamide slab gels and polyvinylidene difluoride membranes (Atto Co., Tokyo, Japan) were also used. Arachidonic acid, *N*-(2-cyclohexyloxy-4-nitrophenyl) methanesulfonamide (NS-398), ovine anti-COX-1 monoclonal antibody, human anti-COX-2 monoclonal antibody, and an enzyme-linked immunosorbent assay kit for PGE2 were obtained from Cayman Chemical Co. (Ann Arbor, MI). All other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

### Western blotting

Approval for the study was obtained from the Ethical Committee of St Marianna University. The purpose of the study was explained to all the participating volunteers, who gave written consent to the use of their samples for research. Western blotting was performed by a modification of the previously described method (Kawai et al 1998). Peripheral venous blood was collected from the healthy volunteers and mononuclear cells were prepared as described previously (Kawai et al 1989). The cells were plated onto plastic plates ($2.5 \times 10^6$ cells mL$^{-1}$) containing RPMI-1640 medium with 0.5% FBS and PC-SM (at a final concn of 100 U mL$^{-1}$ and 100 $\mu$g mL$^{-1}$, respectively), and were incubated at 37°C in a 5% $CO_2$ atmosphere for 2 h. Non-adherent cells were then removed by washing the plates three times with serum-free RPMI-1640 medium, and the adherent cells were used as monocytes. The monocytes were incubated with or without 10 $\mu$g mL$^{-1}$ LPS for 20 h, followed by lysis in 25 mM Tris-HCl (pH 7.5), 2 mM EDTA, 1% Tween-20, and 1 mM phenylmethylsulfonyl fluoride, and then centrifugation at 10 000 $g$ for 10 min at 4°C. The supernatants were collected and the protein content was determined using the DC Protein Assay with bovine serum albumin as the standard. Cell lysates containing 40 $\mu$g of protein were subjected to sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on 10% (w/v) acrylamide slab gels under reducing conditions. The proteins were then electro-transferred to polyvinylidene difluoride membranes. After blocking with 5% powdered skim-milk, the membranes were reacted with an anti-COX-1 monoclonal antibody at a concentration of 50 $\mu$g mL$^{-1}$ or with an anti-COX-2 antibody at 1 $\mu$g mL$^{-1}$. Immunoreactive bands were visualized with biotinylated anti-mouse immunoglobulin G (IgG), streptavidin-peroxidase conjugate, and 3-amino-9-ethylcarbazole as the substrate (HistoScan).

### COX-1 assay system

Monocytes prepared by the method described above were used directly for the assay of COX-1 activity. Cells were washed in RPMI-1640 medium without PC-SM or
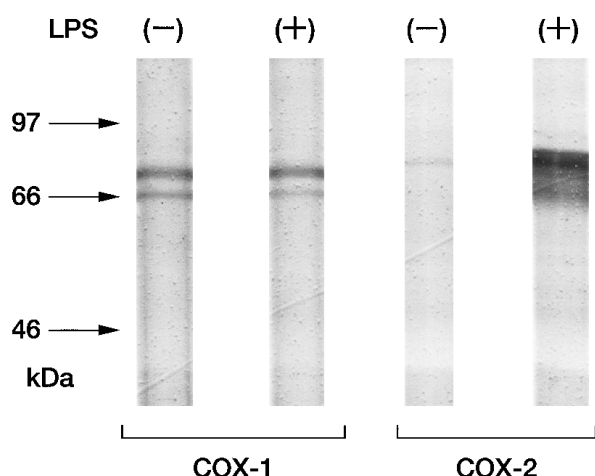
**Figure 1** Western blotting analysis of cyclooxygenase 1 (COX-1) and 2 (COX-2) protein expression by human monocytes. Cell lysates containing 40 $\mu$g of protein were subjected to 10% SDS-PAGE. Proteins transferred to polyvinylidene difluoride membranes were treated with specific mouse monoclonal antibodies against ovine COX-1 or human COX-2, and were visualized using biotinylated anti-mouse IgG, streptavidin-peroxidase conjugate, and 3-amino-9-ethylcarbazole as the substrate.

**Table 1** IC50 values ($\mu$M) of various non-steroidal anti-inflammatory drugs (NSAIDs) for human monocyte cyclooxygenase 1 (COX-1) and 2 (COX-2).

| NSAID | n | COX-1 | COX-2 | COX-1/COX-2 ratio |
|---|---|---|---|---|
| Celecoxib | 4 | $82 \pm 36$ | $6.8 \pm 5.4$ | 12 |
| Diclofenac | 10 | $0.076 \pm 0.018$ | $0.026 \pm 0.010$ | 2.9 |
| Etodolac | 4 | $> 100$ | $53 \pm 45$ | $> 1.9$ |
| Ibuprofen | 8 | $12 \pm 1$ | $80 \pm 52$ | 0.15 |
| Indometacin | 9 | $0.0090 \pm 0.0012$ | $0.31 \pm 0.20$ | 0.029 |
| Meloxicam | 4 | $37 \pm 11$ | $6.1 \pm 3.8$ | 6.1 |
| 6-MNA | 6 | $149 \pm 25$ | $230 \pm 16$ | 0.65 |
| NS-398 | 7 | $125 \pm 29$ | $5.6 \pm 2.6$ | 22 |
| Piroxicam | 3 | $47 \pm 18$ | $25 \pm 6$ | 1.9 |
| Rofecoxib | 4 | $> 100$ | $25 \pm 18$ | $> 4.0$ |
| S-2474 | 4 | $> 100$ | $8.9 \pm 4.8$ | $> 11$ |
| SC-560 | 3 | $0.0048 \pm 0.0026$ | $1.4 \pm 0.3$ | 0.0034 |

IC50 data are mean $\pm$ s.e., of n experiments.

FBS and were then incubated at 37°C with various NSAIDs dissolved in dimethylsulfoxide (DMSO) or with the vehicle alone. The final concentration of DMSO was 0.1% in every sample. After incubation for 30 min, 3 $\mu$M arachidonic acid was added to each well and COX-1 enzyme activity was assessed by measuring

PGE2 production over 15 min. After the specified period, the culture medium was centrifuged at 1500 *g* for 3 min at 4°C and the concentration of PGE2 in the supernatant was measured using an assay kit. In this COX-1 assay system, the basal PGE2 concentration of control supernatant without NSAIDs ranged from 2.4 to 9.8 ng mL$^{-1}$. Both intra- and interassay coefficients of variation for measurement of the PGE2 concentration were less than 10%.

## COX-2 assay system

For the COX-2 assay, some of the mononuclear cells were plated in RPMI-1640 medium with 0.5% FBS, and then 300 $\mu$M aspirin was added to irreversibly block the constitutive expression of COX-1. After 2 h incubation, plates were washed three times to remove non-adherent cells and any residual aspirin. The adherent cells (monocytes) were incubated for 20 h at 37°C with 10 $\mu$g mL$^{-1}$ LPS in RPMI-1640 medium containing 5% FBS and PC-SM. Subsequently, the cells were washed three times in RPMI-1640 medium without PC-SM or FBS. Then the inhibition assay of de-novo induced COX-2 was performed by incubating the cells at 37°C with various NSAIDs dissolved in DMSO or with the vehicle alone. After 30 min, 3 $\mu$M arachidonic acid was added to each well and COX-2 enzyme activity was assessed by measuring PGE2 production over 15 min. After this period, the culture medium was centrifuged at 1500 *g* for 3 min at 4°C and the concentration of PGE2 in the supernatant was measured using an assay kit. In this LPS-stimulated COX-2 assay system, the basal concentration of PGE2 in control supernatant without NSAIDs ranged from 0.3 to 2.8 ng mL$^{-1}$.

## Data analysis

The PGE2 concentration in each supernatant was measured in duplicate. Monocytes from 14 volunteers were used for evaluation of COX-1 and COX-2 inhibition curves. Experiments were performed 3–10 times for each NSAID. The 50% inhibitory concentration (IC50) of each NSAID was calculated using linear regression analysis from the reduction in PGE2 produced by different concentrations of the test drug compared with the control preparation. Results are expressed as the mean $\pm$ s.e. of several experiments. The ratios of the mean IC50 values of COX-1 and COX-2 were calculated. To evaluate the relative potency of COX-1
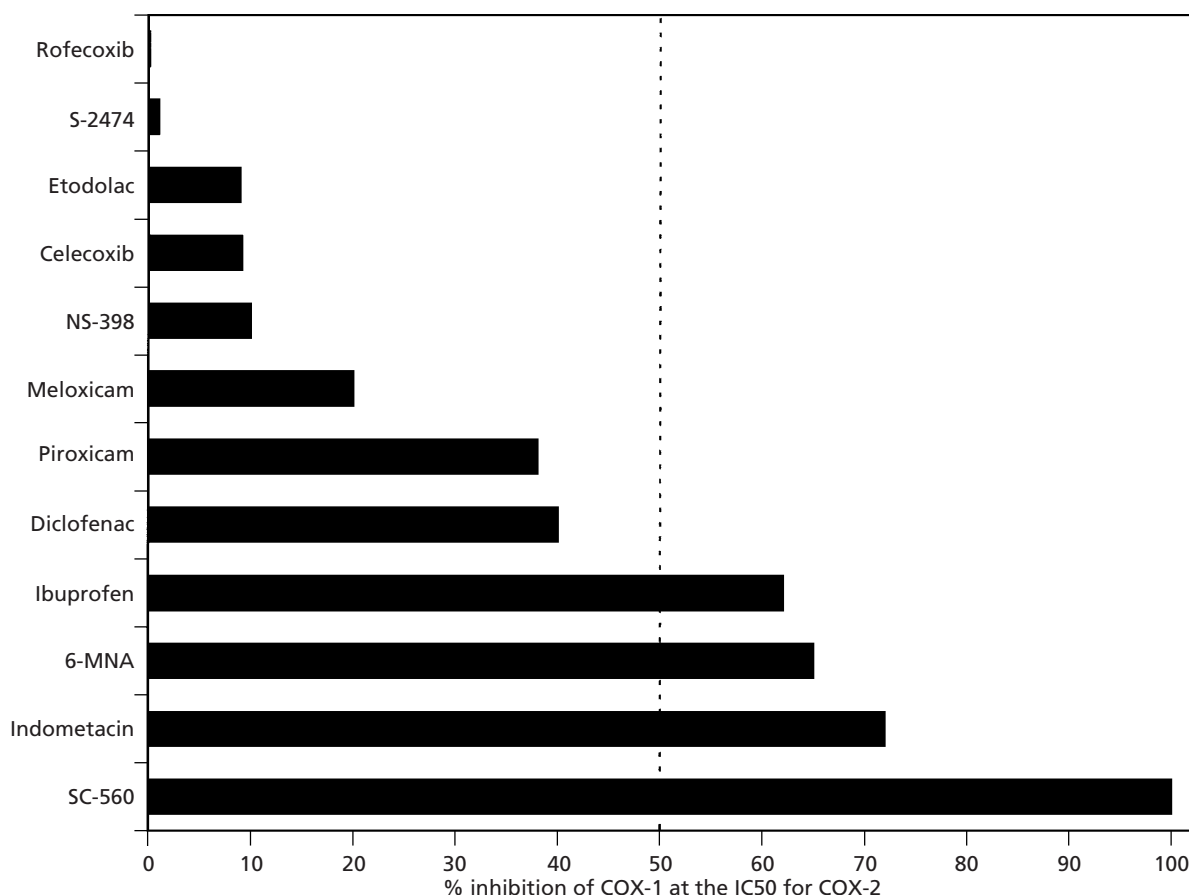
**Figure 2**   The percentage inhibition of COX-1 at the IC50 for COX-2. The dotted line indicates equal inhibitory activity (i.e. 50% inhibition of both COX-1 and COX-2).

versus COX-2 inhibition by the NSAIDs, we determined the percentage inhibition of COX-1 activity at the IC50 concentration of each drug for COX-2.

## Results

### Western blotting of COX-1 and COX-2

In human monocytes without LPS treatment, the anti-COX-1 antibody recognized a major band of approximately 70 kDa, which was considered to be COX-1 protein (Figure 1). A weak band of approximately 66 kDa was also observed. There was little enhancement of COX-1 protein expression after LPS treatment. The anti-COX-2 antibody did not recognize a band in cells without LPS stimulation. However, after exposure of cells to 10 $\mu$g mL$^{-1}$ LPS for 20 h, the COX-2 protein band of approximately 70 kDa was intensely stained.

### Inhibitory effect of various NSAIDs on COX-1 and COX-2

Table 1 summarizes the IC50 (mean $\pm$ s.e.) values of the various NSAIDs for COX-1 activity and LPS-stimulated COX-2 activity in human monocytes. The ratios of the IC50 values of COX-1 and COX-2 for each NSAID are also shown. The rank order of relative potency for inhibition of COX-1 by these NSAIDs was SC-560, indometacin, diclofenac, ibuprofen, meloxicam, piroxicam, celecoxib, NS-398, and 6-MNA. The IC50 values of etodolac, rofecoxib, and S-2474 for COX-1 were all greater than 100 $\mu$M. The rank order of relative potency for inhibition of COX-2 by the NSAIDs was diclofenac, indometacin, SC-560, NS-398, meloxicam, celecoxib, S-2474, rofecoxib, piroxicam, etodolac, ibuprofen, and 6-MNA. Since the IC50 values of etodolac, rofecoxib, and S-2474 for COX-1 could not be measured, we determined the extent of COX-1 inhibition by each NSAID at the IC50 value for COX-2. This allowed us to assess

the relative selectivity of COX-1 versus COX-2 inhibition for all the NSAIDs and the results are shown in Figure 2.

## Discussion

In this study, we established a simple assay system using human monocytes to measure the inhibitory effect of NSAIDs on human COX-1 and COX-2 activity. This assay should be suitable for clinical use. Previous studies on COX selectivity have been performed using extracted native enzymes (Mitchell et al 1994; Yamazaki et al 1997) or recombinant enzymes (Laneuville et al 1994; Glaser et al 1995). However, it has been suggested that assays using intact cells are a more effective method of screening for COX selectivity (Kawai 1998). In addition, Morita et al (1995) have reported that intracellular COX-1 and COX-2 show different locations in murine 3T3 cells, indicating that enzyme assays might not reflect in-vivo cellular conditions.

Previous studies employing intact cells to assess selectivity for human COX have used the following cell types: cos-1 cells (Laneuville et al 1994) or Chinese hamster ovarian cells transfected with human COX-1 or COX-2 expressing genes (Riendeau et al 1997); static and ligand-stimulated human whole blood cells (Glaser et al 1995; Brideau et al 1996; Riendeau et al 1997; Cryer & Feldman 1998; Warner et al 1999); human whole blood cells and interleukin-1-stimulated A549 cells (Warner et al 1999); human platelets and interleukin-1-stimulated synovial cells (Kawai et al 1998); and human platelets and ligand-stimulated mononuclear cells (Grossman et al 1995; Laufer et al 1999). Thus, all of the previous cell assay systems, including our former method, have used different types of cells for measuring COX-1 and COX-2 inhibition or have employed a mixed cell system. This fact may explain the variability of the COX-1/COX-2 IC50 ratios reported by different authors. In contrast, our present system was designed to use a single type of cell (normal human monocytes) for the assay of both COX-1 and COX-2. We confirmed that unstimulated human monocytes only expressed COX-1, and also confirmed that LPS-stimulated monocytes mostly expressed COX-2 when COX-1 was inhibited by pretreatment with aspirin. The ratio of the IC50 values for COX-1 and COX-2 can be used as an indication of the COX-2 selectivity of a particular NSAID. Since we made the experimental conditions for the COX-1 and COX-2 assays as similar as possible, a COX-1/COX-2 IC50 ratio of more than 1 indicates preferential COX-2 selectivity.

To elucidate the relative potency of COX-1 inhibition by these NSAIDs, the extent of COX-1 inhibition by the individual drugs at the IC50 of COX-2 was determined. The mean percentage inhibition of COX-1 by rofecoxib, S-2474, etodolac, celecoxib, NS-398 and meloxicam was less than 20 % at the IC50 for COX-2, indicating weaker COX-1 inhibition. S-2474 is a novel antiarthritic agent that can suppress interleukin-1 production. It was also a selective inhibitor of COX-2 in our assay system and in another study (Inagaki et al 2000). In contrast, SC-560 was reported to be a selective COX-1 inhibitor by Smith et al (1998) and we also found that SC-560 was selective for COX-1.

Selective COX-2 inhibitors seem to cause a lower incidence of severe gastrointestinal side-effects. In our study, rofecoxib, S-2474, etodolac, celecoxib, NS-398, and meloxicam all showed high selectivity for COX-2, whereas SC-560 showed high selectivity for COX-1. Endoscopic gastrointestinal complications related to treatment with rofecoxib (Lanza et al 1999), etodolac (Lanza et al 1987), celecoxib (Simon et al 1999), and meloxicam (Lipscomb et al 1998) were decreased in normal volunteers and in patients with rheumatoid arthritis. The cumulative incidence of confirmed upper gastrointestinal events in patients with osteoarthritis and rheumatoid arthritis who were treated with rofecoxib (Bombardier et al 2000), celecoxib (Silverstein et al 2000), and meloxicam (Distel et al 1996) was also decreased. Our new assay systems may contribute to the prediction of the gastrointestinal effects of new NSAIDs.

## References

Anderson, G. D., Hauser, S. D., McGarity, K. L., Bremer, M. E., Isakson, P. C., Gregory, S. A. (1996) Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J. Clin. Invest.* **97**: 2672–2679

Appleton, I., Tomlinson, A., Willoughby, D. A. (1994) Inducible cyclooxygenase (COX-2): a safer therapeutic target? *Br. J. Rheumatol.* **33**: 410–412

Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., Day, R., Ferraz, M. B., Hawkey, C. J., Hochberg, M. C., Kvien, T. K., Schnitzer, T. J. (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. *N. Engl. J. Med.* **343**: 1520–1528

Brideau, C., Kargman, S., Liu, S., Dallob, A. L., Ehrich, E. W., Rodger, I. W., Chan, C.-C. (1996) A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. *Inflamm. Res.* **45**: 68–74

Crofford, L. J., Wilder, R. L., Ristimäki, A. P., Sano, H., Remmers, E. F., Epps, H. R., Hla, T. (1994) Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1$\beta$, phorbol ester, and corticosteroids. *J. Clin. Invest.* **93**: 1095–1101

Cryer, B., Feldman, M. (1998) Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *Am. J. Med.* **104**: 413–421

Distel, M., Mueller, C., Bluhmki, E., Fries, J. (1996) Safety of meloxicam: a global analysis of clinical trials. *Br. J. Rheumatol.* **35** (Suppl. 1): 68–77

Emery, P. (1996) Clinical implications of selective cyclooxygenase-2 inhibition. *Scand. J. Rheumatol.* **25**: 23–28

Flower, R., Gryglewski, R., Herbaczynska-Cedro, K., Vane, J. R. (1972) Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nat. New Biol.* **238**: 104–106

Glaser, K., Sung. M.-L., O'Neill, K., Belfast, M., Hartman, D., Carlson, R., Kreft, A., Kubrak, D., Hsiao, C.-L., Weichman, B. (1995) Etodolac selectively inhibits human prostaglandin G/H synthase 2 (PGHS-2) versus human PGHS-1. *Eur. J. Pharmacol.* **281**: 107–111

Grossman, C. J., Wiseman, J., Lucas, F. S., Trevethick, M. A., Birch, P. J. (1995) Inhibition of constitutive and inducible cyclooxygenase activity in human platelets and mononuclear cells by NSAIDS and Cox 2 inhibitors. *Inflamm. Res.* **44**: 253–257

Inagaki, M., Tsuri, T., Jyoyama, H., Ono, T., Yamada, K., Kobayashi, M., Hori, Y., Arimura, A., Yasui, K., Ohno, K., Kakudo, S., Koizumi, K., Suzuki, R., Kato, M., Kawai, S., Matsumoto, S. (2000) Novel antiarthritic agents with 1,2-isothiazolidine-1, 1-dioxide (γ-sultam) skeleton: cytokine suppressive dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase. *J. Med. Chem.* **43**: 2040–2048

Kawai, S. (1998) Cyclooxygenase selectivity and the risk of gastrointestinal complications of various non-steroidal anti-inflammatory drugs: a clinical consideration. *Inflamm. Res.* **47**: S102–S106

Kawai, S., Brandon, D. D., Loriaux, D. L., Chrousos, G. P. (1989) Interaction of RU 486, a glucocorticoid and progestin antagonist with human circulating mononuclear leukocytes. *Am. J. Med. Sci.* **298**: 167–171

Kawai, S., Nishida, S., Kato, M., Furumaya, Y., Okamoto, R., Koshino, T., Mizushima, Y. (1998) Comparison of cyclooxygenase-1 and -2 inhibitory activities of various nonsteroidal anti-inflammatory drugs using human platelets and synovial cells. *Eur. J. Pharm.* **347**: 87–94

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**: 12 866–12 872

Laneuville, O., Breuer, D. K., Dewitt, D. L., Hla, T., Funk, C. D., Smith, W. L. (1994) Differential inhibition of human prostaglandin endoperoxide H synthases-1 and -2 by nonsteroidal anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.* **271**: 927–934

Lanza, F., Rack, M. F., Lynn, M., Wolf, J., Sanda, M. (1987) An endoscopic comparison of the effects of etodolac, indomethacin, ibuprofen, naproxen, and placebo on the gastrointestinal mucosa. *J. Rheumatol.* **14**: 338–341

Lanza, F. L., Rack, M. F., Simon, T. J., Quan, H., Bolognese, J. A., Hoover, M. E., Wilson, F. R., Harper, S. E. (1999) Specific inhibition of cyclooxygenase-2 with MK-0966 is associated with less gastroduodenal damage than either aspirin or ibuprofen. *Aliment. Pharmacol. Ther.* **13**: 761–767

Laufer, S., Zechmeister, P., Klein, T. (1999) Development of an in-vitro test system for the evaluation of cyclooxygenase-2 inhibitors. *Inflamm. Res.* **48**: 133–138

Lipscomb, G. R., Wallis, N., Armstrong, G., Rees, W. D. W. (1998) Gastrointestinal tolerability of meloxicam and piroxicam: a double-blind placebo-controlled study. *Br. J. Clin. Pharmacol.* **46**: 133–137

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 antiinflammatory and nonulcerogenic. *Proc. Natl Acad. Sci. USA* **91**: 3228–3232

Mitchell, J. A., Akarasereenont, P., Thiemermann, C., Flower, R. J., Vane, J. R. (1994) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc. Natl Acad. Sci. USA* **90**: 11 693–11 697

Morita, I., Schindler, M., Regier, M. K., Otto, J. C., Hori, T., DeWitt, D. L., Smith, W. L. (1995) Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J. Biol. Chem.* **270**: 10 902–10 908

O'Neill, G. P., Ford-Hutchinson, A. W. (1993) Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett.* **330**: 156–160

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)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). *J. Med. Chem.* **40**: 1347–1365

Riendeau, D., Percival, M. D., Boyce, S., Brideau, C., Charleson, S., Cromlish, W., Ethier, D., Evans, J., Falgueyret, J. -P., Ford-Hutchinson, A. W., Gordon, R., Greig, G., Gresser, M., Guay, J., Kargman, S., Léger, S., Mancini, J. A., O'Neill, G., Ouellet, M., Rodger, I. W., Thérien, M., Wang, Z., Webb, J. K., Wong, E., Chan, C. -C., et al (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *Br. J. Pharmacol.* **121**: 105–117

Silverstein, F. E., Faich, G., Goldstein, J. L., Simon, L. S., Pincus, T., Whelton, A., Makuch, R., Eisen, G., Agrawal, N. M., Stenson, W. F., Burr, A. M., Zhao, W. W., Kent, J. D., Lefkowith, J. B., Verburg, K. M., Geis, G. S. (2000) Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. *JAMA* **284**: 1247–1255

Simon, L. S., Weaver, A. L., Graham, D. Y., Kivitz, A. J., Lipsky, P. E., Hubbard, R. C., Isakson, P. C., Verburg, K. M., Yu, S. S., Zhao, W. W., Geis, G. S. (1999) Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial. *JAMA* **282**: 1921–1928

Smith, C. J., Zhang, Y., Koboldt, C. M., Muhammad, J., Zweifel, B. S., Shaffer, A., Talley, J. J., Masferrer, J. L., Seibert, K., Isakson, P. C. (1998) Pharmacological analysis of cyclooxygenase-1 in inflammation. *Proc. Natl Acad. Sci. USA* **95**: 13 313–13 318

Vane, J. R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* **231**: 232–235

Vane, J. R., Botting, R. M. (1996) Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol.* **25** (Suppl. 102): 9–21

Warner, T. D., Giuliano, F., Vojnovic, I., Bukasa, A., Mitchell, J. A., Vane, J. R. (1999) Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc. Natl Acad. Sci. USA* **96**: 7563–7568

Xie, W., 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. USA* **88**: 2692–2696

Yamazaki, R., Kawai, S., Matsuzaki, T., Kaneda, N., Hashimoto, S., Yokokura, T., Okamoto, R., Koshino, T., Mizushima, Y. (1997) Aceclofenac blocks prostaglandin $E_2$ production following its intracellular conversion into cyclooxygenase inhibitors. *Eur. J. Pharmacol.* **329**: 181–187

Yamazaki, R., Kawai, S., Matsumoto, T., Matsuzaki, T., Hashimoto, S., Yokokura, T., Okamoto, R., Koshino, T., Mizushima, Y. (1999) Hydrolytic activity is essential for aceclofenac to inhibit cyclooxygenase in rheumatoid synovial cells. *J. Pharmacol. Exp. Ther.* **289**: 676–681