Cyclooxygenase-1 and Cyclooxygenase-2 Selectivity of Widely Used Nonsteroidal Anti-Inflammatory Drugs

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PURPOSE: Both isoforms of cyclo-oxygenase, COX-1 and COX-2, are inhibited to varying degrees by all of the available nonsteroidal anti-inflammatory drugs (NSAIDs). Because inhibition of COX-1 by NSAIDs is linked to gastrointestinal ulcer formation, those drugs that selectively inhibit COX-2 may have less gastrointestinal toxicity. We measured the extent to which NSAIDs and other anti-inflammatory or analgesic drugs inhibit COX-1 and COX-2 in humans.

SUBJECTS AND METHODS: Aliquots of whole blood from 16 healthy volunteers were incubated *ex vivo* with 25 antiinflammatory or analgesic drugs at six concentrations ranging from 0 (control) to 100 μM (n = 5 for each). Blood was assayed for serum-generated thromboxane B_2 synthesis (COX-1 assay) and for lipopolysaccharide-stimulated prostaglandin E_2 synthesis (COX-2 assay). In addition, gastric biopsies from the same volunteers were incubated with each drug *ex vivo* and mucosal prostaglandin E_2 synthesis measured.

RESULTS: Inhibitory potency and selectivity of NSAIDs for COX-1 and COX-2 activity in blood varied greatly. Some NSAIDs (eg, flurbiprofen, ketoprofen) were COX-1 selective, some (eg, ibuprofen, naproxen) were essentially nonselective, while others (eg, diclofenac, mefenamic acid) were COX-2 selective. Inhibitory effects of NSAIDs on gastric prostaglandin E_2 synthesis correlated with COX-1 inhibitory potency in blood ($P < 0.001$) and with COX-1 selectivity ($P < 0.01$), but not with COX-2 inhibitory potency. Even COX-2 "selective" NSAIDs still had sufficient COX-1 activity to cause potent inhibitory effects on gastric prostaglandin E_2 synthesis at concentrations achieved in vivo.

CONCLUSION: No currently marketed NSAID, even those that are COX-2 selective, spare gastric COX activity at therapeutic concentrations. Thus, all NSAIDs should be used cautiously until safer agents are developed. **Am J Med. 1998;104: 413–421.** ©1998 by Excerpta Medica, Inc.

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX), the rate-limiting enzyme for synthesis of prostaglandins. Although inhibition of COX may underlie the beneficial anti-inflammatory, analgesic, and antipyretic effects of NSAIDs, simultaneous COX inhibition in the gastrointestinal mucosa often results in gastric and intestinal ulcers, which may have serious consequences. Indeed, all COX-inhibiting NSAIDs currently available by prescription in the United States carry a warning about life-threatening gastrointestinal ulcers.

The recent discovery that there are two COX isoforms, COX-1 and COX-2, that are variably expressed in different tissues raised the possibility that the therapeutic effect of NSAIDs could be separated from their toxic gastrointestinal effects (1–9). COX-1 is expressed constitutively in most tissues throughout the body, including the gastrointestinal mucosa. COX-2 is expressed at low levels in most cells, including the normal human stomach and intestine (10,11). Unlike COX-1, however, expression of COX-2 can be up-regulated at inflammatory sites by cytokines and bacterial products such as lipopolysaccharide. Thus, a NSAID that inhibits COX-2 while sparing COX-1 has the potential to be anti-inflammatory yet nontoxic to the gastrointestinal tract.

Although currently available NSAIDs inhibit both COX-1 and COX-2, many appear to inhibit one isoform to a greater extent than the other (12–19). Most data on functional differences in COX isoform inhibition (selectivity) of NSAIDs have come from studies in animals or in isolated cells. No studies have compared the effects of various NSAIDs on the human gastrointestinal mucosa.

In this study, we investigated the COX-selectivity of several NSAIDs in healthy men and women. First, we measured the effects of the drugs in whole blood, using assays that specifically detect COX-1 or COX-2 inhibition. Second, we studied the effects of the same drugs in the stomach by measuring ex vivo rates of prostaglandin synthesis in human gastric mucosal biopsies exposed to these agents. Finally, we compared inhibitory effects of the drugs on COX-1 and COX-2 in blood with their ability to inhibit prostaglandin synthesis in the stomach.

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Table 1. Drugs Tested

Non-aspirin NSAIDs
•Diclofenac (Voltaren)
•Etodolac (Lodine)
•Fenoprofen (Nalfon)
•Flurbiprofen (Ansaid)
•Ibuprofen (Motrin)
•Indomethacin (Indocin)
•Ketoprofen (Orudis)
•Ketorolac (Toradol)
•Mefenamic Acid (Ponstel)
•Naproxen (Naprosyn)
•Oxaprosin (Daypro)
•Piroxicam (Feldene)
•Tolmetin (Tolectin)
Pro-drug NSAIDs
•Sulindac (Clinoril)
•Nabumetone (Relafen)
Metabolite
•6-MNA (metabolite of nabumetone)
Acetylated salicylate
•Aspirin
Non-acetylated Salicylates
•Salicylic acid
•Salsalate (Disalcid)
•Valeryl salicylate
•Bismuth subsalicylate (Pepto-Bismol)
Analgesic
•Acetaminophen (Tylenol)
Steroid
•Dexamethasone (Decadron)
Investigational
•NS-398
•Nimesulide

MATERIALS AND METHODS

Prior to enrollment of study subjects, this study was approved by the Human Studies Subcommittee of the Dallas Department of Veterans Affairs Medical Center.

We enrolled 16 nonsmoking healthy volunteers (6 men and 10 nonpregnant women) between the ages of 23 and 46 years. None had a history of peptic ulcer, gastrointestinal surgery (except for appendectomy), NSAID use in the past 2 weeks, chronic upper gastrointestinal symptoms, or use of histamine H_2-receptor antagonists, proton pump inhibitors, misoprostol, or sucralfate. Each subject was seronegative for *Helicobacter pylori* infection (FlexSure *Hp*; SmithKline Diagnostics, Inc. San Jose, California). Subjects were scheduled for visits on study days 1 and 10. At both visits, health and medication histories were confirmed, venous blood was obtained for COX-1 and COX-2 whole blood assays, after which each subject underwent endoscopy with mucosal biopsy. During the interval between study days 1 and 10, no medications were dispensed or allowed.

We studied 25 anti-inflammatory, analgesic, and other drugs of interest (Table 1). Most were NSAIDs, including 6-methoxy napthalene acetic acid (6-MNA), the active metabolite of nabumetone. (Sulindac sulfide, the active metabolite of sulindac was not available.) Other drugs included four nonacetylated salicylates, the corticosteroid dexamethasone, acetaminophen, and two experimental anti-inflammatory agents (NS-398 and nimesulide). We purchased from the manufacturer powdered formulations of 22 of the drugs, specifically ibuprofen, ketorolac, 6-methoxy napthalene acetic acid, nimesulide, NS-398, and valeryl salicylate (from Cayman Chemical Co., Ann Arbor, Michigan); and acetylsalicylic acid (aspirin), acetaminophen, dexamethasone, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, mefenamic acid, naproxen, nabumetone, piroxicam, tolmetin, salicylic acid, and sulindac (from Sigma Chemical Co., St. Louis, Missouri). Powdered formulations of bismuth subsalicylate (Proctor and Gamble, Cincinnati, Ohio), oxaprosin (G.D. Searle Pharmaceuticals, Chicago, Illinois), and salsalate (3M Pharmaceuticals, St. Paul, Minnesota) were donated by the manufacturer.

For each drug, stock solutions were made by mixing powdered drug with dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Missouri). Then, six concentrations of drug (100, 10, 1, 0.1, 0.01 and 0 μM) were prepared by mixing the appropriate amount of stock solution either with 10 mM phosphate buffer saline (Sigma Chemical Co., St. Louis, Missouri) for COX-1 and COX-2 whole blood assays or with 150 mM Tris Buffer (Trizma Hydrochloride; Sigma Chemical Co., St. Louis, Missouri) for gastric mucosal COX assays. New solutions were made on each study day just prior to obtaining whole blood or gastric mucosal biopsies.

COX-1 whole blood assay. The measurement of thromboxane B_2 synthesis from platelets following blood coagulation is a specific test for COX-1 activity (18,19). Platelets constitutively express COX-1. In response to endogenous thrombin formation (ie, clot formation) platelet COX-1 is maximally stimulated to produce thromboxane A_2, which is converted to thromboxane B_2. Thromboxane B_2 production reaches a plateau 1 hour after coagulation.

On each study day, siliconized glass tubes were organized into four sets of six tubes so that each subject's blood sample would be exposed to the same four drugs as his or her gastric mucosal biopsies. After venipuncture, 4-mL aliquots of whole blood were immediately transferred to tubes preloaded with freshly made study drug solution so that final drug concentrations in each set of 6 tubes were 100, 10, 1, 0.1, 0.01, and 0 μM. All tubes were briefly and gently vortexed to distribute drug thoroughly

and then allowed to clot, initially for 45 minutes at 37°C and then for 2 additional hours at room temperature. At the end of the 2-hour 45-minute incubation period, indomethacin was added to the tube at a final concentration of 30 μM, to terminate thromboxane B_2 synthesis. This was followed by centrifugation at 2,000*g* for 10 minutes at room temperature. One mL serum from each tube was then stored at −70°C until thromboxane B_2 was measured by radioimmunoassay, as previously described (18). Mean (± SD) baseline thromboxane B_2 synthesis in whole blood at 2 hours 45 minutes was 236.0 ± 105.4 ng/mL.

COX-2 whole blood assay. The measurement of prostaglandin E_2 production from monocytes and macrophages in whole blood following stimulation with lipopolysaccharide is a specific test for COX-2 activity. Twenty-four hours after incubation of whole blood with lipopolysaccharide, 95% to 99% of COX activity is COX-2 (18,19). Thus, prostaglandin E_2 measured 24 hours after lipopolysaccharide stimulation should almost exclusively reflect COX-2 activity.

First, 1.5 mL polypropylene microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania) were preloaded with the same drugs and concentrations used for that subject's COX-1 and gastric mucosal assays. Next, whole blood was collected by venipuncture into heparinized vacuum tubes, pooled, and aliquoted into 24, 500-μL samples so that the same final six drug concentrations would be achieved as with the other assays. Lipopolysaccharide (50 μg *E Coli* serotype (127:B8; Sigma Chemical Co., St. Louis, Missouri) was added to each tube, briefly vortexed, and then incubated for 24 hours at 37°C for induction of COX-2. Tubes were then centrifuged at 12,000*g* for 5 minutes, and 100 μL of the resulting plasma was mixed with 400 μl of methanol for extraction of prostaglandins and precipitation of proteins. Supernatants were then stored at −70°C until they were assayed for prostaglandin E_2 by radioimmunoassay using a kit (Prostaglandin E_2 [I^{125}], Biotrak radioimmunoassay system; Amersham Life Sciences, Arlington Heights, Illinois). Mean (± SD) lipopolysaccharide-stimulated prostaglandin E_2 synthesis in whole blood at 24 hours was 127.8 ± 105.5 ng/mL.

Endoscopy and gastric mucosal biopsy. Each subject, after an overnight fast, received topical pharyngeal anesthesia with tetracaine hydrochloride (Pontocaine; Winthrop Pharmaceuticals, New York, New York), followed by light sedation with intravenous midazolam (Versed; Roche Laboratories, Nutley, New Jersey). Using an Olympus endoscope (Olympus Corporation, New York, New York), the gastroduodenal mucosa was visually inspected. No subject had any gastroduodenal erosion or ulcer at either endoscopy. Following mucosal inspection, 24 mucosal biopsies were taken from the greater curvature of the gastric body. Each gastric mucosal biopsy was immediately placed in a study drug incubation solution.

Gastric mucosal prostaglandin E_2 ex vivo synthesis assay. The ability of the gastric mucosa to generate prostaglandins (prostaglandin synthesis rate) estimates total gastric COX activity (COX-1 plus any COX-2). To measure ex vivo prostaglandin synthesis rates, gastric mucosal biopsies were immediately placed in 1.5 mL polypropylene microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania) that contained 1 mL study drug incubation solution. The 24 biopsies from each endoscopic session were allocated to four different drugs at six concentrations per drug as directed by a previously determined drug allocation schedule. This schedule was designed so that gastric mucosal prostaglandin synthesis was assessed five times for each of the six concentrations of every drug, and so that each time a drug concentration was tested (n = 5), the incubated gastric mucosal biopsy would be from a different study subject.

Ex vivo gastric mucosal prostaglandin synthesis was performed by a modification of a method previously described by our laboratory (20). Briefly, incubation tubes containing biopsies were weighed, and mucosal biopsy weight was determined after subtraction of the original tube weight. Each mucosal biopsy was then finely minced with scissors for 15 seconds, allowed to incubate after mincing for 3 minutes, and then briefly centrifuged at 14,000*g* for 15 seconds to pellet tissue. Since endoscopic biopsies were placed in solution at varying times, original incubation solutions were then discarded by aspiration and replaced with 1 mL identical fresh solutions at timed intervals. Next, biopsies were briskly vortexed for 3 minutes (to generate and liberate prostaglandins). After 15 seconds of centrifugation, indomethacin (10^{-5} M) was added to the supernatant to stop further prostaglandin synthesis. Supernatants were then stored at −70°C until ready for measurement of prostaglandin E_2 by radioimmunoassay (21). Prostaglandin synthesis is expressed as nanograms of prostaglandin E_2 synthesized per gram-minute of tissue incubation. Mean (± SD) gastric mucosal prostaglandin E_2 synthesis at baseline was 190.2 ± 318.7 ng/g-min.

Calculation of drug IC_{50}. For each study drug, a dose-response curve was constructed by plotting the six drug concentrations (x axis, logarithmic scale) against mean percent inhibition for each group of five subjects (y axis) of thromboxane B_2 (for blood COX-1 assay), lipopolysaccharide-induced prostaglandin E_2 (for blood COX-2 assay), or gastric prostaglandin E_2 (for gastric mucosal COX assay). Next, the concentration of study drug that inhibited mean COX activity by 50% (IC_{50}) was calculated by computer-assisted extrapolation. Specifically, a computer-generated curve of concentration versus mean

Table 2. Concentration of Drug (IC_{50}) that Inhibited 50% of Cyclooxygenase (COX) Activity in Blood and in Gastric Mucosa (μM)

Drug	COX-1 in Blood (Rank)	COX-2 in Blood (Rank)	Gastric Mucosa (Rank)
Ketoprofen	0.11 (1)	0.88 (8)	0.08 (2)
Indomethacin	0.21 (2)	0.37 (7)	0.85 (11)
Diclofenac	0.26 (3)	0.01 (1)	0.23 (4)
Ketorolac	0.27 (4)	0.18 (6)	0.33 (6)
Flurbiprofen	0.41 (5)	4.23 (13)	0.23 (5)
Tolmetin	1.08 (6)	2.25 (11)	3.50 (16)
Mefenamic acid	1.94 (7)	0.16 (4)	0.70 (10)
Piroxicam	2.68 (8)	2.11 (10)	0.87 (12)
Fenoprofen	2.73 (9)	14.03 (17)	0.17 (3)
Aspirin	4.45 (10)	13.88 (16)	0.03 (1)
Ibuprofen	5.90 (11)	9.90 (14)	0.70 (9)
Nimesulide	10.48 (12)	0.18 (5)	1.49 (13)
Oxaprosin	14.58 (13)	36.67 (23)	2.62 (14)
Etodolac	19.58 (14)	2.47 (12)	3.20 (15)
NS-398	21.93 (15)	0.92 (9)	100.00 (18)
6-MNA	31.01 (16)	19.84 (19)	0.48 (7)
Naproxen	32.01 (17)	28.19 (22)	0.52 (8)
Valeryl salicylate	32.64 (18)	0.04 (2)	>100.00 (21)
Nabumetone	33.57 (19)	20.83 (20)	20.09 (17)
Sulindac	41.26 (20)	24.94 (21)	>100.00 (19)
Acetaminophen	42.23 (21)	10.69 (15)	>100.00 (23)
Dexamethasone	59.95 (22)	0.13 (3)	>100.00 (25)
Bismuth subsalicylate	75.24 (23)	37.50 (24)	>100.00 (22)
Salicylic acid	>100.00 (24)	14.08 (18)	>100.00 (20)
Salsalate	>100.00 (25)	39.90 (25)	>100.00 (24)

6-MNA = 6-methoxy napthalene acetic acid.

percent inhibition (for each group of five subjects) was constructed for each drug. Using the formula for the slope of the line between the two drug doses that bracketed (above and below) 50% inhibition, the IC_{50} was calculated. For some study drugs, COX activity was inhibited by less than 50% at the maximal concentration tested (100 μM). In such instances the IC_{50} is reported as greater than 100 μM.

Statistical Analysis

To determine correlations between drug IC_{50}s in whole blood assays and gastric mucosal assays, Spearman's rank-correlation coefficient and Pearson's coefficient of correlation were used (22). *P* values less than 0.05 were considered statistically significant.

RESULTS

For the 25 study drugs, IC_{50}s for inhibition in the COX-1 exclusive whole blood assay (platelet-generated serum thromboxane B_2 synthesis) are ranked in Table 2. All but two of the drugs (salicylic acid and salsalate) inhibited COX-1 at the concentrations tested in this study. Ketoprofen had the lowest IC_{50} (0.11 μM) and was thus the most potent COX-1 inhibitor in blood. The range in potency of COX-1 inhibition varied at least 1,000-fold among the 25 drugs.

All 25 drugs tested also inhibited COX-2 activity in blood (Table 2), with potency varying approximately 4,000-fold. The NSAID, diclofenac, had the most potent COX-2 inhibitory effect in blood (IC_{50} = 0.01 μM), even more potent than dexamethasone. Some NSAIDs (eg, fenoprofen, naproxen, oxaprosin and 6-MNA, the active metabolite of nabumetone) were less potent COX-2 inhibitors in blood than acetaminophen. Aspirin and acetaminophen had similar effects on COX-2 in blood (Table 2), contrasting with aspirin's nearly 10-fold greater potency on COX-1 (Table 2).

COX selectivity in blood. Rankings of the 25 drugs in Table 3 reflect their relative selectivity for inhibition of COX-1 or COX-2 in blood. A ratio of COX-2 IC_{50} to

Table 3. Ratio of Concentration of Drug (IC_{50}) that Inhibited 50% of Cyclooxygenase-1 (COX-1) Activity to the IC_{50} for COX-2 Activity in Blood

Rank	Drug	Ratio*
1	Flurbiprofen	10.27
2	Ketoprofen	8.16
3	Fenoprofen	5.14
4	Aspirin	3.12
5	Oxaprosin	2.52
6	Tolmetin	2.09
7	Indomethacin	1.78
8	Ibuprofen	1.69
9	Naproxen	0.88
10	Piroxicam	0.79
11	Ketorolac	0.68
12	6-MNA	0.64
13	Nabumetone	0.62
14	Sulindac	0.61
15	Bismuth subsalicylate	0.50
16	Salsalate	0.29†
17	Acetominophen	0.25
18	Salicylic acid	0.13†
19	Etodolac	0.12
20	Mefenamic acid	0.08
21	Diclofenac	0.05
22	NS-398	0.042
23	Nimesulide	0.017
24	Dexamethasone	0.002
25	Valeryl salicylate	0.001

* Ratios >1 indicate that a drug is more COX-1 selective; ratios <1 indicate that a drug is more COX-2 selective.

† Since the actual IC_{50} for COX-1 in blood was >100 μM, a value of 100 μM was used in the calculation of this ratio. Therefore, these ratios represent a maximum possible ratio of IC_{50} COX-2/IC_{50} COX-1 in blood.

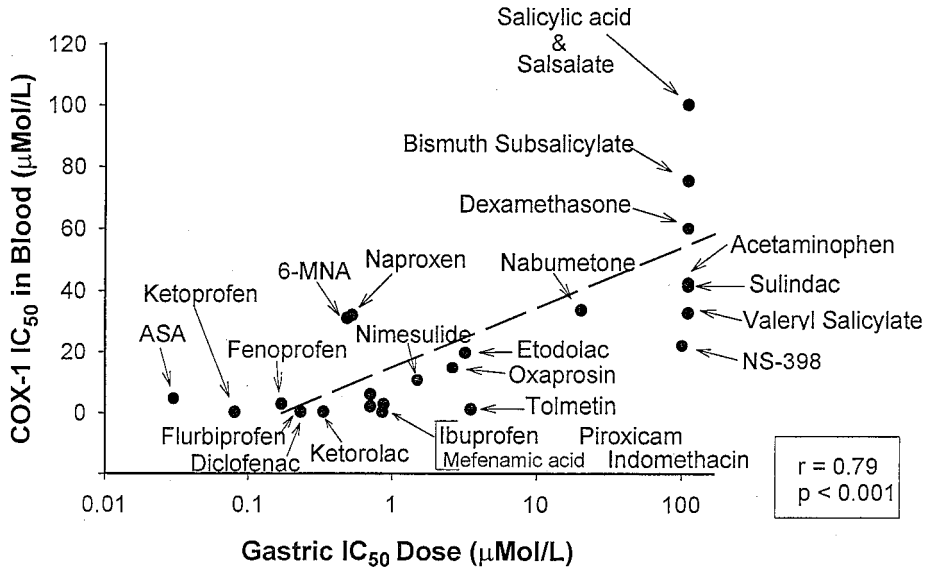

Figure 1. Correlation of gastric IC_{50} with COX-1 IC_{50} in blood for the 25 drugs. Rank order correlation was 0.79 ($P < 0.001$) and Pearson correlation coefficient was 0.75 ($P < 0.001$). Drugs with an IC_{50} in gastric mucosa or blood >100 μM are arbitrarily plotted at 100 μM in this and subsequent figures. ASA = acetysalicylic acid (aspirin); MNA = methoxy napthalene acetic acid.

COX-1 IC_{50} close to 1.0 indicates nearly equal selectivity. Selectivity among the 25 drugs varied approximately 10,000-fold. A few NSAIDs were COX-2 selective in blood, particularly diclofenac (IC_{50} ratio = 0.05, indicating 20-fold selectivity for COX-2), mefenamic acid (12.5-fold), and etodolac (8.5-fold). The two experimental NSAIDs, nimesulide (59-fold) and NS-398 (24-fold), were particularly COX-2 selective. Dexamethasone and the experimental compound valeryl salicylate were the most COX-2 selective agents tested.

COX effects in gastric mucosa. Potency on gastric mucosal COX inhibition varied by at least 300-fold among the 25 drugs (Table 2). Aspirin was the most potent gastric COX inhibitor. The four non-acetylated salicylates (valeryl salicylate, salicylic acid, salicylsalicylic acid, and bismuth subsalicylate), acetaminophen, and dexamethasone had no measurable effects on COX activity in the stomach. As shown in Figure 1, the IC_{50} for gastric mucosal COX correlated significantly correlated with the IC_{50} for COX-1 in blood ($P < 0.001$). As illustrated in

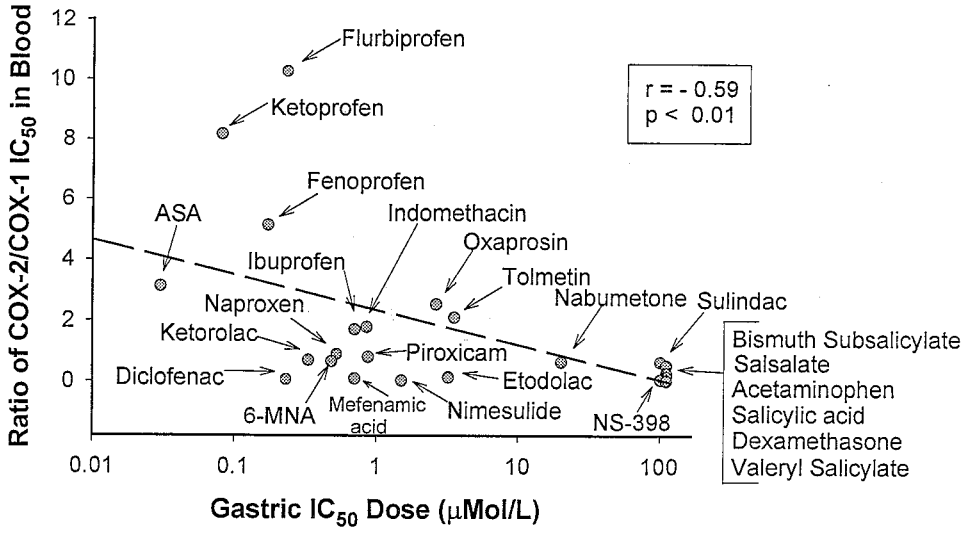

Figure 2. Correlation of gastric IC_{50} with ratio of COX-2 IC_{50}/COX-1 IC_{50} in blood for the 25 drugs. Rank order correlation was −0.59 ($P < 0.01$) and Pearson correlation coefficient was −0.54 ($P < 0.01$). ASA = acetysalicylic acid (aspirin); MNA = methoxy napthalene acetic acid.

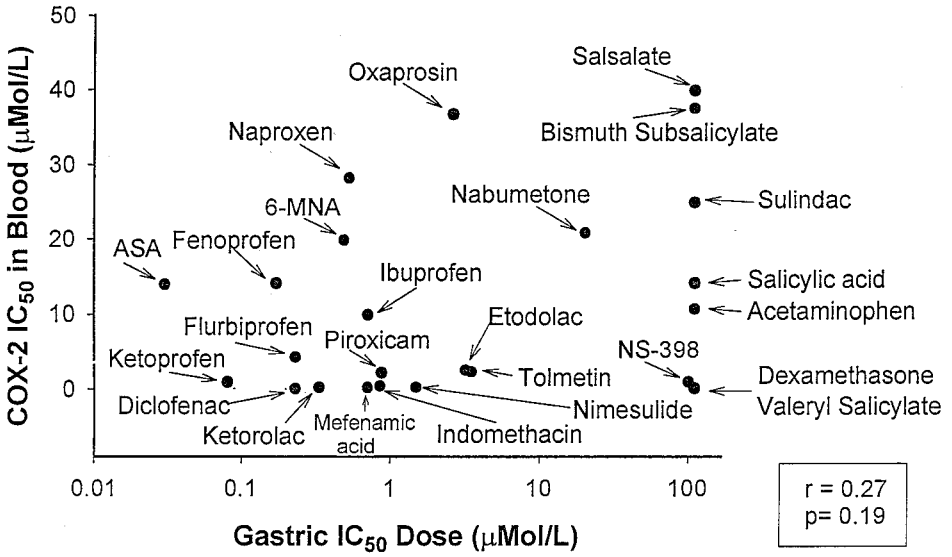

Figure 3. Correlation of gastric IC_{50} with COX-2 IC_{50} in blood for the 25 drugs. Rank order correlation was 0.26 and Pearson correlation coefficient was 0.27 (both $P = 0.19$). ASA = acetysalicylic acid (aspirin); MNA = methoxy napthalene acetic acid.

Figure 2, the IC_{50} for gastric COX also correlated with the degree of COX-1 selectivity in blood ($P < 0.01$). In contrast, the IC_{50} for gastric mucosal COX inhibition did not correlate with the IC_{50} for COX-2 in blood (Figure 3).

DISCUSSION

We have conducted a comprehensive analysis of the COX-1 versus COX-2 effects of NSAIDs in healthy volunteers. Our results regarding COX-selectivity can be compared with those of previous reports using nonhuman cells (14,16). We found that two agents, indomethacin and piroxicam, that were quite COX-1 selective for murine COX, were essentially nonselective for COX in human blood (Table 3). Likewise, 6-MNA was sevenfold selective for murine COX-2, but only onefold to twofold for human COX-2. Using bovine endothelial cells (COX-1) and murine macrophages stimulated by lipopolysaccharide (COX-2), Mitchell et al (16) analyzed the selectivity of five of the NSAIDs that we examined. They found aspirin (166-fold), indomethacin (60-fold), and ibuprofen (15-fold) to be quite COX-1 selective, while these drugs were only twofold to threefold COX-1 selective in human blood (16). Naproxen was COX nonselective in both Mitchell's study and in ours. Diclofenac was also nonselective in the previous study, while we found diclofenac to be 20-fold COX-2 selective in human blood. Differences in COX selectivity among these various studies are probably related to species differences in the COX proteins.

The healthy gastrointestinal mucosa in humans predominantly expresses COX-1, with very little COX-2 expression (10,11). Gastrointestinal mucosal COX-1 is responsible for catalyzing the synthesis of mucosal-protective prostaglandins from their precursor, arachidonic acid (4,6,21). NSAIDs that are specific or very highly selective inhibitors of COX-2 might have few gastrointestinal side effects because mucosal synthesis of prostaglandins would be preserved. Of the currently available NSAIDs that we examined, diclofenac was the most potent and the most selective inhibitor of COX-2 in whole blood. Even though diclofenac was 20 times more selective for COX-2 than for COX-1, this drug still had sufficient COX-1 inhibitory activity in the stomach to be a potent inhibitor of gastric mucosal prostaglandin synthesis. Dexamethasone, on the other hand, one tenth as potent as diclofenac as a COX-2 inhibitor, was 25 times more selective than diclofenac for COX-2 inhibition in blood. Moreover, this corticosteroid had essentially no gastric mucosal COX inhibition at concentrations up to 100 μM, perhaps explaining why corticosteroid medications given without NSAIDs are not a risk factor for gastroduodenal ulcers (23). The observation that a highly selective and potent COX-2 inhibitor such as dexamethasone had no demonstrable effect on gastric COX supports previous observations (10,11) that there is very little COX-2 activity within the human stomach. The correlation we found between COX-1 potency of NSAIDs in blood and the gastric mucosa also reinforces the concept that the healthy stomach mainly, if not exclusively, expresses COX-1.

In addition to the potency and selectivity of a drug for COX-1 or COX-2, the administered dose is another important feature that must be taken into consideration when assessing overall potential gastrointestinal mucosal effects. To evaluate whether the drug concentrations used

Table 4. Serum Nonsteroidal Antiinflammatory Drug (NSAID) Concentrations at Usual Therapeutic Dosage

NSAID	Normal Dose (mg/day)	Mean Serum Concentration (μM)	IC_{50} for Gastric COX Inhibition (μM)*	Reference
Aspirin (high dose)	1200–5200	111.0†	0.03	(25)
(low dose)	81–325	4.9–18.6†	0.03	‡
Diclofenac	150–200	6.1†	0.23	(26)
Etodolac	600–1200	83.5	3.20	(24)
Fenoprofen	800–3200	89.5	0.17	(24)
Flurbiprofen	200–300	53.2	0.23	(27)
Ibuprofen (high dose)	1200–3200	111.0†	0.70	(28)
(low dose)	800–1200	38.8†	0.70	(28)
Indomethacin	75–200	1.4	0.85	(25)
Ketoprofen	100–300	9.4	0.08	(24)
Ketorolac	10–40	8.0†	0.33	(24)
Mefenamic acid	500	82.9	0.70	(24)
Naproxen	500–1000	1.3	0.52	(29)
Nimesulide	100–200	14.6	1.49	(30)
Oxaprosin	1200	681.9	2.62	(24)
Piroxicam	20	16.6	0.87	(24)
Tolmetin	1200–1800	126.9	3.50	(24)
Sulindac sulfide§	300–400‖	14.6	not tested	(31)
6-MNA¶	1000–2000‖	155.4	0.48	(32,33)

* From Table 5.
† Single-dose serum concentration.
‡ Cryer and Feldman, unpublished information.
§ Active metabolite of sulindac (prodrug).
‖ Dosed as prodrug.
¶ Active metabolite of probumetone (prodrug).
6-MNA = 6-methoxy napthalene acetic acid

in these experiments and the calculated IC_{50}s were comparable with the usual serum concentrations seen with therapeutic dosing, we compiled pharmacokinetic data for NSAIDs from a review of the available literature (Table 4) (24–33). For every drug for which data could be compared, their expected therapeutic serum concentrations was well above their IC_{50} for gastric COX inhibition. For example, the peak concentration of diclofenac in blood after a single oral dose is 6.1 μM, more than 25 times higher than the IC_{50} in gastric mucosa. Although it is not known to what extent, and for how long, gastric COX must be blocked to increase the risk of ulcer formation, all of the NSAIDs that we studied appear capable of producing gastrointestinal mucosal prostaglandin depletion at customary doses.

Aspirin irreversibly acetylates COX at a serine moiety near the active site of the enzyme (34–36). In the systems we tested, aspirin's ability to acetylate COX allowed it to be a modestly potent inhibitor of COX-1 in blood and the most potent gastric COX inhibitor, while the nonacetylated salicylates had relatively few effects in either COX-1 system. These ex vivo comparisons of the effects of salsalate (salicylsalicylic acid) and aspirin in the human stomach confirm our previous in vivo observations. In humans administered these two drugs at doses that produced identical serum salicylate concentrations, aspirin markedly reduced gastroduodenal mucosal prostaglandin content, while the effects of salsalate were indistinguishable from placebo (37).

COX inhibition by aspirin via acetylation appears to play less of a role in COX-2 inhibition than in COX-1 inhibition, since valeryl salicylate (pentanoyl rather than acetyl substitution) was a very potent and selective inhibitor of COX-2, while aspirin only modestly inhibited COX-2 in blood. Interestingly, aspirin acetylates active serine sites in both COX-1 and COX-2 (34–36). In the case of COX-1, aspirin acetylation reduces COX activity by preventing arachidonate binding to the COX active site (34,35). With COX-2, however, acetylation does not block substrate binding (36).

Two prodrug NSAIDs were evaluated in our study, sulindac and nabumetone. Only after absorption are these prodrugs converted to active metabolites that have antiinflammatory activity. Shortly after absorption, sulindac is reversibly reduced to sulindac sulfide, its active metabolite (31). Sulindac sulfide is systemically distributed and inhibits COX throughout the body, including the stomach. Sulindac sulfide has been reported to be 500 times

more potent than sulindac as a COX inhibitor (25); unfortunately, sulindac sulfide was not available to us at the time of the study. When sulindac was introduced, it was stated to have a low incidence of gastrointestinal side effects (38). With longer periods of clinical experience, however, gastrointestinal complications with sulindac have been similar to those of other NSAIDs (39).

Nabumetone is a prodrug that is converted to its active metabolite 6-MNA (32,33) after absorption. When 6-MNA and nabumetone were compared in the current study, they were equally potent as inhibitors of COX-1 and COX-2 in blood, and both were only minimally COX-2 selective. This probably occurred because nabumetone is rapidly converted to 6-MNA in blood (32). However, this conversion may not occur in the gastric mucosa, since 6-MNA was 40 times more potent a gastric COX inhibitor than nabumetone. Thus, the 6-MNA metabolite of nabumetone may lead to gastrointestinal mucosal prostaglandin depletion and predispose to ulcer formation. Chronic nabumetone administration in humans is associated with gastrointestinal ulceration (24), but the incidence may be somewhat lower than with other NSAIDs (40).

Because of the limitations that are inherent in the estimation of IC_{50}s through extrapolation, relative ranking of drugs' COX effects may not be as precise as suggested by our listings. For example, a minor change in the extrapolated estimates of COX-1 inhibitory IC_{50}s for diclofenac and ketorolac could result in a switching of their positions in the rank list. The COX effects of our study drugs should be viewed as relative ranges of selectivity or potency rather than absolute rankings. Another limitation is that COX responses to NSAIDs in our ex vivo biopsy specimens may differ from in vivo responses. Finally, it is possible that COX isoform distribution or COX responses to NSAIDs in healthy subjects may differ from those in nonhealthy patients such as those with peptic ulcer disease or other inflammatory conditions, or who are taking other medications.

Newer drugs that may be more COX-2 selective than currently available drugs are in various stages of research and development (41,42). Such agents may prove to be effective anti-inflammatory agents that spare the gastrointestinal tract from injury. However, as seen from the comparisons in the current study, even a high degree of COX-2 selectivity does not guarantee freedom from gastric COX inhibition. Gastric mucosal COX inhibition is affected by a combination of COX selectivity, COX potency, and drug pharmacokinetics. The ideal NSAID should be prescribed in doses that are high enough to be anti-inflammatory, antipyretic, or analgesic, but not so high that they result in serum and gastrointestinal mucosal concentrations that substantially reduce gastrointestinal mucosal COX-1 activity and prostaglandin synthesis. None of the currently marketed NSAIDs, even those that are COX-2 selective, have these gastrointestinal-sparing features. NSAIDs should, therefore, continue to be used cautiously until safer agents are developed.

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