Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase

(cytokines/arachidonic acid/prostanoids/inflammation/mitogen/aspirin-like drugs)

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ABSTRACT Constitutive cyclooxygenase (COX-1; prostaglandin-endoperoxide synthase, EC 1.14.99.1) is present in cells under physiological conditions, whereas COX-2 is induced by some cytokines, mitogens, and endotoxin presumably in pathological conditions, such as inflammation. Therefore, we have assessed the relative inhibitory effects of some nonsteroidal antiinflammatory drugs on the activities of COX-1 (in bovine aortic endothelial cells) and COX-2 (in endotoxin-activated J774.2 macrophages) in intact cells, broken cells, and purified enzyme preparations (COX-1 in sheep seminal vesicles; COX-2 in sheep placenta). Similar properties of aspirin, indomethacin, and ibuprofen against the broken cell and purified enzyme preparations indicated no influence of species. Aspirin, indomethacin, and ibuprofen were more potent inhibitors of COX-1 than COX-2 in all models used. The relative potencies of aspirin and indomethacin varied only slightly between models, although the IC₅₀ values were different. Ibuprofen was more potent as an inhibitor of COX-2 in intact cells than in either broken cells or purified enzymes. Sodium salicylate was a weak inhibitor of both COX isoforms in intact cells and was inactive against COX in either broken cells or purified enzyme preparations. Diclofenac, BW 755C, acetylsalicylic acid, and naproxen were approximately equipotent inhibitors of COX-1 and COX-2 in intact cells. BF 389, an experimental drug currently being tested in humans, was the most potent and most selective inhibitor of COX-2 in intact cells. Thus, there are clear pharmacological differences between the two enzymes. The use of such models of COX-1 and COX-2 activity will lead to the identification of selective inhibitors of COX-2 with presumably less side effects than present therapies. Some inhibitors had higher activity in intact cells than against purified enzymes, suggesting that pure enzyme preparations may not be predictive of therapeutic action.

Cyclo-oxygenase (COX; prostaglandin-endoperoxide synthase, EC 1.14.99.1) converts arachidonic acid to prostaglandin (PG) H₂, which is then further metabolized by other enzymes to various PGs, prostacyclin, and thromboxanes (1). COX exists in at least two isoforms with similar molecular weights (~70 kDa). COX-1 is expressed constitutively and was first characterized, purified, and cloned from sheep vesicular glands (2–7). Activation of COX-1 leads, for instance, to the production of prostacyclin, which when released by the endothelium is antithrombogenic (8) and by the gastric mucosa is cytoprotective (9). COX-2 is induced in cells exposed to proinflammatory agents, including cytokines (10), mitogens (11) and endotoxin (12, 13). Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the activity of COX, a property that accounts for their shared therapeutic and side effects (14). Thus, the ability of NSAIDs to inhibit COX-2 may well explain their therapeutic utility as antiinflammatory drugs, whereas inhibition of COX-1 may explain their unwanted side effects, such as gastric and renal damage.

After establishing that bovine aortic endothelial cells in culture contain COX-1 and that endotoxin-activated J774.2 macrophages contain COX-2, we have investigated the inhibitory effects of some NSAIDs on the activity of COX-1 and COX-2 in whole cells and broken cells and in purified enzyme preparations. Our results show that NSAIDs have different profiles of inhibition of COX-1 and COX-2 in a range of models.

METHODS

Cell Culture. Murine macrophages (J774.2; The European Collection of Animal Cell Culture, Salisbury, U.K.) were grown in 96-well culture plates with Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 4 mM l-glutamine. Bovine aortic endothelial cells (BAEC) were cultured from fresh bovine aortae as described (15) and seeded onto 96-well culture plates.

Intact Cells. COX-1. BAEC were incubated for 30 min with aspirin (0.1 ng/ml to 1 mg/ml), indomethacin (0.1 ng/ml to 1 mg/ml), ibuprofen (0.1 to 1 mg/ml), sodium salicylate (0.1 to 100 μg/ml), diclofenac (0.1 ng/ml to 100 μg/ml), naproxen (0.1 ng/ml to 1 mg/ml), acetylsalicylic acid (0.1 mg/ml), BW 755C (0.1 ng/ml to 100 μg/ml), or a nonacacid NSAID, BF 389 (0.1 mg/ml to 1 mg/ml; ref. 37). Arachidonic acid (30 μM) was then added, and the cells were incubated for a further 15 min at 37°C. The medium was then removed, and radioimmunoassay (16) was used to measure the formation of 6-keto-PGF₁α, PGE₂, thromboxane B₂, or PGF₂α. Antibodies to 6-keto-PGF₁α, PGE₂, thromboxane B₂, and PGE₂ were obtained from Sigma. Tritiated 6-keto-PGF₁α, PGE₂, thromboxane B₂ or PGF₂α were obtained from Amersham.

COX-2. Cultured J774.2 macrophages were treated with endotoxin at 1 μg/ml for 12 hr to induce COX-2. Culture medium was then changed, and one of the NSAIDs was added (see above) for 30 min at 37°C. Arachidonic acid (30 μM) was then added, and the cells were incubated for a further 15 min at 37°C. The medium was removed and analyzed by radioimmunoassay as above. The inhibitory effects of NSAIDs on COX were measured in at least nine separate determinations (wells) on at least 3 different experimental days.

Cell Viability. Cell suspension, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan as described (17). Treatment of J774.2

Abbreviations: BAEC, bovine aortic endothelial cells; COX, cyclooxygenase; NSAIDs, nonsteroidal antiinflammatory drugs; PG, prostaglandin.

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macrophages with lipopolysaccharide at 1 µg/ml for 12 hr did not significantly inhibit cell viability.

**Broken Cell Preparation.** COX-1. BAEC were cultured in T175 flasks until confluent. The cells were washed and scraped into ice-cold phosphate-buffered saline (pH 7.4). The cells were then centrifuged at 1000 × g for 10 min, and the cell pellet was homogenized with a glass Teflon homogenizer in 50 mM Tris buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 50 µM peptatin A, and 0.2 mM leupeptin. The broken cells (150 µg of protein) were incubated at 37°C in the presence of one of the NSAIDs (see above) for 30 min. Arachidonic acid (30 µM) was added, and the incubation was continued for a further 15 min. The reaction was then stopped by boiling. The samples were centrifuged at 10,000 × g for 30 min, and 6-keto-PGF$_{1α}$ was measured in the supernatant as an indicator of COX-1 activity.

COX-2. J774.2 macrophages were cultured in T175 flasks until confluent. Endotoxin at 1 µg/ml was added for 12 hr, after which time the cells were washed and homogenized; the effects of NSAIDs on COX-2 activity were assayed as for COX-1 in the broken-cell preparations of BAEC.

**Purified Enzymes.** Purified COX-1 and COX-2 were obtained from Cayman Chemicals (Ann Arbor, MI) and were gifts from Biofor (Waverly, PA). Enzyme activity was measured by the conversion of [14C]arachidonic acid to PGE$_2$ after separation by thin layer chromatography (TLC). A solution (100 µl) of aspirin (150 µg), sodium salicylate, ibuprofen, or sodium salicylate was added to a reaction mixture containing 6.6 µM arachidonic acid (saturating substrate concentration), together with [14C]arachidonic acid (100,000 disintegrations per minute), and was made up to a final volume of 1 ml with 50 mM Tris buffer (pH 8 at 37°C) containing the cofactors glutathione (5 mM), epinephrine (5 mM), and hematin (1 µM). The reaction was initiated by the addition of 10 units of enzyme. Samples were incubated in a shaking water bath at 37°C for 10 min (during which period the reaction was linear), after which the reaction was stopped by adding 30 µl of 1 M HCl. One milliliter of saturated NaCl solution was added to each sample followed by 1.5 ml of ethyl acetate. All samples were mixed in a Vortex and centrifuged at 1500 rpm for 10 min (575 × g). The ethyl acetate layer (1 ml) was removed to a separate tube and concentrated under a stream of nitrogen.

PGs were separated by TLC. Each sample was redissolved in 30 µl of chloroform/methanol, 2:1 (vol/vol), and 20 µl was applied onto a glass-backed silica gel plate. The TLC plate was developed for ~90 min at room temperature in a solvent consisting of the upper phase of ethyl acetate/trimethylpentane/acetate/water, 110:50:20:100 (vol/vol).

For detection of 14C-labeled PGs, autoradiography was performed by placing the plate in contact with x-ray film for 12 hr. The film was then developed and fixed. The PGE$_2$ band was located on the plate and scraped off, and the absolute radioactivity was estimated by scintillation counting.

**Immunoblot (Western Blot) Analysis.** BAEC or endotoxin-activated (1 µg/ml; 12 hr) J774.2 macrophages cultured in T175 flasks were washed with phosphate-buffered saline (pH 7.4) and incubated for 10 min with 2–3 ml of extraction buffer [50 mM Tris/10 mM EDTA/1% (vol/vol) Triton X-100/1 mM phenylmethylsulfonyl fluoride/50 µM peptatin A/0.2 mM leupeptin] while being gently shaken. The cell extract was then boiled for 10 min with gel-loading buffer [50 mM Tris/10% (wt/vol) SDS/10% (vol/vol) glycercol/10% 2-mercaptoethanol/2 mg of bromphenol blue per ml] in a ratio of 1:1 (vol/vol). The samples were loaded onto gradient gels (4–12% Tris glycine; Novex, British Biotechnology, Oxford) and separated by electrophoresis. After transfer to nitrocellulose, the blot was primed with a selective antibody raised to ovine COX-1 developed in rabbits (a gift from K. Wu Houston) or a rabbit antibody raised to murine COX-2 (Cayman Chemical). The blot was then incubated with an anti-rabbit IgG developed in sheep and linked to alkaline phosphatase conjugate, and the blot was developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium.

**Materials and Statistical Analysis.** All compounds used were obtained from Sigma unless otherwise stated. BF 389 was a gift from Biofor and BW 755C was a gift from Wellcome Research Laboratories. Data were analyzed by using Student’s unpaired t test and taking a P value of <0.05 as significant.

**RESULTS**

**Characterization of COX-1 and COX-2 Activities.** The basal release of eicosanoids from untreated J774.2 macrophages was below the limits of detection, which were 0.3 ng/ml for 6-keto-PGF$_{1α}$, PGE$_2$, thromboxane B$_2$, and PGF$_{2α}$ (n = 6–15). After exposure to endotoxin at 1 µg/ml for 12 hr, the cells released significantly higher amounts (P < 0.05, for all) of 6-keto-PGF$_{1α}$ (1.55 ± 0.19 ng/ml), PGE$_2$ (2.60 ± 0.08 ng/ml), thromboxane B$_2$ (0.87 ± 0.03 ng/ml), and PGF$_{2α}$ (3.46 ± 0.10 ng/ml). BAEC released 6-keto-PGF$_{1α}$ (2.30 ± 0.31 ng/ml; n = 9) and thromboxane B$_2$ (0.43 ± 0.01 ng/ml) but <0.3 ng/ml of PEGF$_2$ or PGF$_{2α}$ (n = 9, for each). Therefore, the formation of 6-keto-PGF$_{1α}$ was used as a common indicator of COX activity in BAEC or endotoxin-activated J774.2 macrophages.

**Characterization of COX Isosforms Present in BAEC or Endotoxin-Activated J774.2 Macrophages.** Antibodies to COX-1 recognized a band of approximately 70 kDa in the extracts of BAEC (Fig. 1A, lane 1) that was not recognized by antibodies to COX-2 (Fig. 1B, lane 1). In contrast, COX-2 protein was present in extracts of endotoxin-activated J774.2 macrophages (Fig. 1B, lane 3) with low levels of COX-1 (Fig. 1A, lane 3), and untreated J774.2 macrophages contained no COX-2 (Fig. 1B, lane 2) and low levels of COX-1 (Fig. 1A, lane 2).

**Effects of NSAIDs on the Activity of Purified COX-1 and COX-2.** In these assays, there was no preincubation of the NSAIDs with the enzyme before adding the substrate. Aspirin, indomethacin, or ibuprofen were much more potent as inhibitors of purified COX-1 than of purified COX-2 (Table 1). Sodium salicylate was inactive against purified COX-1 at concentrations up to 1 mg/ml and produced only a 20% decrease in activity.

**Fig. 1.** BAEC contain COX-1 and endotoxin-activated J774.2 macrophages contain COX-2, as shown by Western blots with polyclonal antibodies to COX-1 (A) or COX-2 (B). Cell extracts of BAEC (lanes 1) contained a protein (~70-kDa molecular mass) that was recognized by antibodies to COX-1 (A) but not by antibodies to COX-2 (B). Low levels of COX-1 or COX-2 protein were detected in untreated J774.2 macrophages (lanes 2). Endotoxin-activated J774.2 macrophages contained a protein (~70-kDa molecular mass) that was recognized by antibodies to COX-2 (lanes 3). A band of ~70 kDa was recognized by COX-1 antibodies in cell extracts from endotoxin-activated J774.2 macrophages. As the COX-1 antibody used cross reacts with COX-2 (10%), this band is at least in part attributed to COX-2 protein (lane 3). Equal amounts of protein were loaded in all lanes (2–3 µg per lane). Similar results were obtained with cell extracts from two separate batches of cells.
inhibition of COX-2 at the maximum concentration used (1 mg/ml). The ratios of activities in favor of COX-1 varied from 42 to 50. In contrast, BF 389 with IC50 values of 4 μg/ml for COX-1 and 8 μg/ml for COX-2 gave a ratio of 2.

Effects of NSAIDs on Broken-Cell Preparations of BAEC and Endotoxin-Activated J774.2 Macrophages. In these experiments, the NSAID was preincubated with the enzyme for 30 min. Aspirin, indomethacin, or ibuprofen inhibited the activity of COX-1 more potently than COX-2 in broken cells (Table 1). The activity of aspirin was similar to that found with purified enzymes, as was that of ibuprofen. However, indomethacin showed IC50 values lower in the broken cells by a factor of 10 than in the purified enzymes. COX-1 activity in broken cells was unaffected by sodium salicylate. COX-2 activity in broken cells was only slightly inhibited (=20%) by the highest concentration of sodium salicylate used (1 mg/ml; Table 1).

Effects of NSAIDs on COX Activity in Whole Cells. Indomethacin was the most potent inhibitor of COX-1 and was some 60 times more potent than against COX-2 (Table 2; Fig. 2B). Aspirin was 166 times more active against COX-1 but was less potent than indomethacin on either isoform. Ibuprofen was 15 times more active against COX-1 than COX-2. Interestingly, the activity of ibuprofen against COX-2 in the intact cells was some 10-fold greater than on the broken cells. Sodium salicylate showed weak activity in both cell preparations but was only 3 times more potent against COX-1 than COX-2. Acetaminophen was also an inhibitor of COX-1 (IC50, 2.7 ± 2.0 μg/ml) and COX-2 (IC50, 0.2 ± 12.0 μg/ml) activity in intact cells. Diclofenac, naproxen, and BW 755C were approximately equipotent inhibitors of COX-1 or COX-2 in intact cells (Table 2). The nonacidic NSAID BF 389 was the most potent inhibitor tested against COX-2 (IC50; 0.03 μg/ml) and also showed the greatest selectivity for COX-2 (ratio 0.2). It was substantially more potent in the intact cells than against the purified enzymes.

**DISCUSSION**

The discovery of an inducible isoform (COX-2) of cyclooxygenase (13) allows a reinterpretation and refinement of the general theory that inhibition of COX activity explains the therapeutic and side effects of the aspirin-like drugs (14). COX-2 is induced in migratory and other cells by inflammatory stimuli (18) presumably through cytokine production. Therefore, it is attractive to suggest that the antiinflammatory actions of the NSAIDs are due to inhibition of COX-2, whereas the unwanted side effects, such as gastric toxicity and nephrotoxicity, are due to inhibition of the constitutive enzyme (COX-1), the products of which help to protect the stomach and the kidney against damage. Here, we explore this hypothesis by testing some NSAIDs on different preparations of COX-1 and COX-2. We show that BAEC contain predominantly COX-1 and that endotoxin-activated J774.2 macrophages contain predominantly COX-2. Using the COX activity in these cells as models for COX-1 and COX-2 or using purified enzymes, we demonstrate that NSAIDs have different relative potencies as inhibitors of COX-1 and COX-2.

There is little evidence to suggest that the potencies of NSAIDs as inhibitors of COX-1 and COX-2 vary from

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**Table 1.** IC50 values for aspirin, indomethacin, ibuprofen, sodium salicylate (salicylate), and BF 389 as inhibitors of COX activity in broken-cell preparations and of purified COX.

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Broken cells</th>
<th>Purified enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COX-1</td>
<td>COX-2</td>
</tr>
<tr>
<td>Aspirin</td>
<td>8</td>
<td>200</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3</td>
<td>160</td>
</tr>
<tr>
<td>Salicylate</td>
<td>Inactive</td>
<td>&gt;1 mg/ml</td>
</tr>
<tr>
<td>BF 389</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

The data on purified enzymes represent the mean of two determinations. Similar results were seen in separate experiments with different batches of COX-1 and COX-2. ND, not determined.

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**Table 2.** IC50 values for NSAIDs on COX-1 and COX-2 activity in intact cells

<table>
<thead>
<tr>
<th>NSAID</th>
<th>IC50, μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.0 ± 0.07</td>
</tr>
<tr>
<td>Acetaminophen*</td>
<td>2.7 ± 2.0</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>BW 755C</td>
<td>0.65 ± 0.26</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Naproxen</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>BF 389</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

The data show the mean ± the SEM for three to five determinations calculated from the means of triplicate determinations. The ratio of the IC50 values for the NSAIDs on COX-2 relative to COX-1 is given in the final column of the table.

*For acetaminophen, IC50 values are shown because 50% inhibition of COX-2 was not achieved at concentrations up to 1 mg/ml.

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**Fig. 2.** The effects of aspirin (A), indomethacin (B), ibuprofen (C), and sodium salicylate (D) on COX activity in BAEC (C) or J774.2 macrophages treated with endotoxin at 1 μg/ml for 12 hr (A). COX activity was measured by the formation of 6-keto-F1α after exposure to exogenous 30 μM arachidonic acid for 10 min. Aspirin and indomethacin (Left) were more potent inhibitors of 6-keto-PGF1α formation from BAEC (COX-1) than from endotoxin-activated J774.2 macrophages (COX-2). Sodium salicylate and ibuprofen (Right) were equipotent inhibitors. Data are expressed as mean ± SEM from 9–15 determinations from at least three separate experimental days.
species to species. Nevertheless, because the two main cell types we have used are from ox (BAEC) and mouse (J774.2), we have compared the effects of four NSAIDs on crude broken-cell preparations with those on purified enzyme preparations from the sheep. On the broken-cell preparations of guinea pig lung (presumably COX-1), aspirin had an IC50 of 7 µg/ml (14). Here we find the IC50 on broken BAEC cells to be 8 µg/ml and on purified sheep COX-1 to be 5 µg/ml. There is, then, good consistency for aspirin as an inhibitor of COX-1 from several different species.

In both crude and purified enzyme preparations (Table 1), aspirin, indomethacin, and ibuprofen were much less active against COX-2 than against COX-1, and the ratios of activity remained remarkably consistent (25–53) between the two assay systems used. Indomethacin was about 10 times more active as an inhibitor of COX activity in broken cell preparations than as an inhibitor of purified COX-1 or COX-2. This may be due to the different experimental protocols used, for indomethacin was preincubated for 30 min with the broken cell preparations but was added simultaneously with arachidonic acid substrate in the purified enzymes.

Our results with purified COX-1 and COX-2 to assess the inhibitory effects of indomethacin (50 times more potent against COX-1 than COX-2) are very similar to those of Meade et al. (ref. 19; indomethacin was 32 times more potent against COX-1 than COX-2). They also found that aspirin was a selective inhibitor of COX-1 using membrane fractions from transfected cells.

Can the effects of NSAIDs on COX-1 explain their unwanted effects? The two strongest inhibitors of COX-1 in all assay systems used, aspirin and indomethacin, are the two NSAIDs that cause the most gastric damage (20). Even though indomethacin is more potent than aspirin as an inhibitor of COX activity in a variety of systems (14, 21) and as an antiinflammatory drug (21, 22), it is also more potent than aspirin as an ulcerogenic agent (see ref. 23). What is more important than the relative potencies of NSAIDs against each other on COX is the ratio of activities on COX-1 and COX-2. Thus, assuming that COX-2 is relevant to inflammation, a more antiinflammatory dose of indomethacin or aspirin will suppress COX-1 activity in the stomach and kidney with a 50-fold greater potency.

Aspirin irreversibly inhibits COX-1 by acetylation of a single serine residue on the enzyme (24). Indomethacin inhibits COX-1 activity by binding to its active site and subsequently rendering it inactive (25), possibly by producing a conformational change in an essential protein radical (26). Although the mechanisms by which aspirin and indomethacin inhibit COX are different, the relative potencies of these drugs were similar in all of the assays used in this study and in those using microsomal preparations from COS-1 transfected cells for assay of COX-1 and COX-2 activity (19).

Ibuprofen, which inhibits COX by substrate competition with arachidonic acid (27), produces less side effects than either aspirin or indomethacin (28). This would not be predicted from the ratios obtained on the enzyme preparations, but in intact cells ibuprofen was at least 5 times more potent than aspirin as an inhibitor of COX-2, whereas the two NSAIDs were equipotent at inhibiting COX-1 activity. These results on intact cells may explain why at equiactive antiinflammatory doses, ibuprofen produces less ulcerogenic side effects than aspirin. However, Meade et al. (19) find that ibuprofen is equipotent as an inhibitor of COX-1 and COX-2 activity in microsomes from transfected COS-1 cells. As the mechanism of action of ibuprofen is based on substrate competition, these differences in the potency of ibuprofen may be due to variations in the accessibility to the enzyme and in the relative concentration of arachidonic acid present in the different assay systems.

Vane (14), using broken-cell preparations of guinea pig lung (COX-1), found salicylate to be much weaker as an inhibitor of COX than aspirin. Here we confirm this observation by showing that aspirin is more potent than salicylate as an inhibitor of COX-1 or COX-2. However, salicylate and aspirin are said to be equally effective antiinflammatory agents leading to the criticism (29) of the theory that inhibition of PG synthesis alone explains the actions of NSAIDs. Do the present results with salicylate help explain this anomaly? Here we show that aspirin is ~100 times more potent than salicylate as an inhibitor of COX-1 in intact cells but only twice as potent as an inhibitor of COX-2. These observations may explain those of Higgs et al. (30) showing that salicylate and aspirin were approximately equipotent inhibitors of COX activity in explants of acutely inflamed tissue (COX-2). However, aspirin was found to be considerably more potent than salicylate as an inhibitor of PGs in the serum (COX-1). Salicylate had negligible inhibitory actions on COX activities in broken cell preparations or purified enzyme. The mechanism of action of salicylate as an inhibitor of COX activity in intact cells may be compounded by its suppression of the induction of COX (31).

Acetaminophen also posed a problem for the original theory of the mechanism of action of NSAIDs, for at therapeutic doses acetaminophen has weak antiinflammatory activity but is a stronger analgesic and antipyretic (32). Flower and Vane (21) found that COX preparations from the brain were more sensitive to acetaminophen than those from the spleen and suggested that there may be different isoforms of COX. We found that acetaminophen inhibited both COX-1 and COX-2 activity in intact cells. However, acetaminophen was less potent as an inhibitor of COX-2 activity than all other NSAIDs tested, with the exception of aspirin and sodium salicylate. The weak inhibitory actions of acetaminophen on COX-2 may explain why this drug has only weak antiinflammatory actions. Perhaps there are other isoforms of COX ("COX-3") in the endothelial cells in the brain that initiate fever by the release of PGs (33).

There is a third group of NSAIDs including BW 755C, diclofenac, and naproxen in which the ratio (IC50 against COX-2/IC50 against COX-1) in intact cells is about 1. The dual COX and lipoxygenase inhibitor BW 755C (34) is an experimental antiinflammatory agent with little or no ulcerogenic activity (9), which fits well with our general hypothesis. Diclofenac and naproxen are less ulcerogenic than aspirin or indomethacin at antiinflammatory doses (ref. 35; see ref. 23).

Interestingly, of all the NSAIDs tested, BF 389, an experimental drug now being tested in man, was the most potent agent against COX-2 in intact cells and also displayed a ratio (0.2) with the greatest selectivity for COX-2. This fits well with toxicological reports on this experimental drug, for BF 389 has little or no gastric ulcerogenicity (36). The fact that BF 389 is more potent in intact cells than on purified enzymes may well be due to the fact that it is concentrated in tissues (37). The results with BF 389 highlight the differences that can be observed between intact cells and purified enzymes. Particularly the inhibition of purified enzymes as a screening method for antiinflammatory activity is a logical starting point, but results from intact cell preparations may well correlate better with biological activity in animals and humans.

Thus, our results support the hypothesis that the side effects of NSAIDs correlate with their ability to inhibit COX-1, while the antiinflammatory (therapeutic) effects of these agents are due to their ability to inhibit COX-2. Inhibition of COX-2 activity may be achieved at several levels in the cascade of events leading to induction of enzyme activity. First, the actions of proinflammatory mitogens can be blocked with receptor antagonists or antibodies. Second, once the cell is activated, the synthesis of COX-2 may be blocked with agents such as glucocorticosteroids (38) or...
salicylates (31). Finally, once COX-2 has been synthesized, selective inhibitors of COX-2 would inhibit the production of proinflammatory prostanooids without affecting, for instance, prostacyclin production by the endothelium (COX-1; Fig. 3). The identification of selective inhibitors of COX-1 and COX-2 will not only provide an opportunity to test this hypothesis, but also lead to advances in the therapy of inflammation. As new compounds become available to inhibit COX-2, the use of aspirin will diminish in inflammation, but expand as an inhibitor of COX-1 in platelets for the prevention of thrombosis.

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Fig. 3. Relationships between the pathways leading to the generation of eicosanoids by COX-1 or COX-2. Under physiological conditions, activation of COX-1 for instance in platelets, endothelium, stomach mucosa, or kidney results in the release of thromboxane A2 (TXA2), prostacyclin (PGI2), or prostaglandin E2 (PGE2). The release of these eicosanoids is selectively inhibited by drugs such as aspirin (I). Inflammatory stimuli release cytokines, such as interleukin 1, which induce the synthesis of COX-2 in cells, such as macrophages, resulting in the release of prostaglandins (PGs). The release of PGs together with proteases and other inflammatory mediators (such as reactive oxygen radicals) results in inflammation. The COX-2 pathway can be interrupted at several levels by antagonists or antibodies to cytokines and mitogens (2), inhibitors of the induction of COX-2 (e.g., glucocorticoids) (3), or selective inhibitors of COX-2 (4).