Nonsteroidal Anti-inflammatory Drugs as Anticancer Agents: Mechanistic, Pharmacologic, and Clinical Issues

Michael J. Thun, S. Jane Henley, Carlo Patrono

Numerous experimental, epidemiologic, and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promise as anticancer agents. NSAIDs restore normal apoptosis in human adenomatous colorectal polyps and in various cancer cell lines that have lost adenomatous polyposis coli gene function. NSAIDs also inhibit angiogenesis in cell culture and rodent models of angiogenesis. Many epidemiologic studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and, to some extent, other cancers. Two NSAIDs, sulindac and celecoxib, have been found to inhibit the growth of adenomatous polyps and cause regression of existing polyps in randomized trials of patients with familial adenomatous polyposis (FAP). However, unresolved questions about the safety, efficacy, optimal treatment regimen, and mechanism of action of NSAIDs currently limit their clinical application to the prevention of polyposis in FAP patients. Moreover, the development of safe and effective drugs for chemoprevention is complicated by the potential of even rare, serious toxicity to offset the benefit of treatment, particularly when the drug is administered to healthy people who have low annual risk of developing the disease for which treatment is intended. This review considers generic approaches to improve the balance between benefits and risks associated with the use of NSAIDs in chemoprevention. We critically examine the published experimental, clinical, and epidemiologic literature on NSAIDs and cancer, especially that regarding colorectal cancer, and identify strategies to overcome the various logistic and scientific barriers that impede clinical trials of NSAIDs for cancer prevention. Finally, we suggest research opportunities that may help to accelerate the future clinical application of NSAIDs for cancer prevention or treatment. [J Natl Cancer Inst 2002;94:252–66]

Several recent reviews (1–3) have summarized the intriguing and accumulating evidence that nonsteroidal anti-inflammatory drugs (NSAIDs) have promise as anticancer drugs. NSAIDs have been shown experimentally to stimulate apoptosis and to inhibit angiogenesis, two mechanisms that help to suppress malignant transformation and tumor growth. Numerous epidemiologic (nonrandomized) studies (4–37) have found that long-term users of aspirin or other NSAIDs have a lower risk of colorectal adenomatous polyps and colorectal cancer than nonusers, although one study has not (38–40). Randomized clinical trials have confirmed that two NSAIDs, the prodrug sulindac (41–43) and the selective cyclooxygenase (COX)-2 inhibitor celecoxib (44), effectively inhibit the growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP). Despite these positive findings, the efficacy and safety of long-term NSAID prophylaxis against colorectal or other cancers remain unproven. While some experts have proposed that there is now sufficient evidence for persons at high risk of large bowel cancer to begin taking low-dose aspirin prophylactically (45), most have not. Health organizations and consensus groups have been appropriately cautious by withholding any recommendation regarding the use of NSAIDs for the prevention or treatment of cancer, except for the use of celecoxib or sulindac to suppress the growth of colorectal adenomatous polyps in patients with FAP (46). Despite enthusiasm about the potential usefulness of NSAIDs, particularly the selective COX-2 inhibitors, as anticancer agents, fundamental questions remain about their safety, efficacy, mechanisms of action, optimal treatment regimens, and contraindications for preventive therapy.

Because of the formidable challenges involved in developing safe and effective drugs for chemoprevention, discussed below, there is continuing need to improve cross-disciplinary communication in planning randomized clinical trials of NSAIDs for chemoprevention. This review combines the perspectives of two epidemiologists (M. J. Thun and S. J. Henley) and a clinical pharmacologist (C. Patrono) to examine the scientific evidence underlying randomized clinical trials of NSAIDs for cancer prevention or treatment. Using the example of aspirin prophylaxis for the prevention of cardiovascular disease, we consider the delicate balance of risks and benefits that complicates primary prevention of cancer. The safety of treatment is of particular concern when large numbers of healthy people must be treated prophylactically for many years to prevent adverse events in a small percentage of those treated. We suggest generic strategies that can improve the benefit–risk balance. We critically review the published experimental, clinical, and epidemiologic evidence regarding NSAIDs and cancer, focusing particularly on colorectal cancer, and suggest research strategies and opportunities that may help to accelerate the future clinical application of NSAIDs for the prevention or treatment of cancer.

Affiliations of authors: M. J. Thun, S. J. Henley, Department of Epidemiology and Surveillance Research, American Cancer Society, Atlanta, GA; C. Patrono, Center of Excellence on Aging, Università degli Studi “G. D’Annunzio,” Chieti, Italy.

Correspondence to: Michael J. Thun, M.D., M.S., Department of Epidemiology and Surveillance Research, American Cancer Society, National Home Office, 1599 Clifton Rd., N.E., Atlanta, GA 30329–4251 (e-mail: mthun@cancer.org).

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BACKGROUND

Challenges of Chemoprevention

At least two constraints make it particularly difficult to develop safe and effective drugs to prevent cancer in average- or low-risk populations. The first is the very low margin for toxicity whenever prophylactic drugs are administered over a long period of time to healthy people who have comparatively low risk of the disease being prevented. Although colorectal cancer accounts for almost 12% of all newly diagnosed cancers in the United States (47), the probability that an individual at average risk will develop colorectal cancer in a given year is low. On average, the cumulative probability of developing colorectal cancer from birth to age 79 years is approximately 4% in men and 3% in women; this probability increases to 5.6% in both sexes over full-life expectancy (48). Thus, in the general population, more than 94% of the people treated prophylactically to prevent colorectal cancer will not benefit from prophylactic treatment unless the benefits extend to other health endpoints. However, the risk of colorectal cancer is considerably higher in certain genetically susceptible subgroups. For example, estimates of lifetime risk, based on studies of high-risk families, are approximately 17% for persons with two affected first-degree relatives, 70% for individuals with genetic mutations associated with hereditary nonpolyposis colon cancer (HNPCC), and more than 95% in persons with FAP (49). However, these high-risk groups contribute only a small fraction of all cases of colorectal cancer.

A second constraint to developing drugs to prevent cancer in average- or low-risk populations concerns the logistic difficulty of studying cancer endpoints, especially those associated with colorectal cancer, in large-scale prevention trials. Because of the low annual risk of developing colorectal cancer in the general population, phase III cancer prevention trials must be much larger than either trials of cardiovascular disease in high-risk populations or therapeutic trials in cancer patients already diagnosed with disease to have adequate statistical power. More than 150 clinical trials have been conducted to assess the efficacy of aspirin as an antiplatelet agent in patients at high risk of cardiovascular disease. Most of these trials enrolled fewer than 3000 patients, and follow-up ranged from 6 months to 4 years (50,51). Trials of similar size and duration have sufficient statistical power to assess the prevention of recurrent colorectal adenomatous polyps in high-risk groups. In contrast, trials of colorectal cancer prevention would require up to 100 times more subjects with 10–20 years of follow-up. Furthermore, trials of both adenomatous polyps and colorectal cancer prevention are logistically more difficult than prevention trials of other cancer sites because they require that study subjects undergo special examinations (i.e., sigmoidoscopy or colonoscopy) before and after the intervention. Consequently, clinical decisions about the efficacy of chemopreventive drugs will need to be based on a smaller number of phase III trials that measure surrogate endpoints in high-risk populations. Decisions about the treatment regimen to be tested in these trials must be based largely on preclinical and epidemiologic evidence, on phase I trials involving 20–80 participants, and on phase II studies of up to several hundred subjects.

Example of aspirin in the prevention of cardiovascular events. Randomized trials of aspirin in the prevention of cardiovascular disease illustrate that the safety of prophylactic treatment is influenced critically by the background risk of the population being treated (50,52,53). Fig. 1 shows that the cardiovascular benefit of aspirin treatment, as measured by the prevention of myocardial infarction (MI), thrombotic stroke, or death from all causes, increases in relation to the annual background risk of these events in the population. Each data point in Fig. 1 is based on the combined data only from published randomized trials of aspirin prophylaxis at less than or equal to 500 mg daily in specified populations (50,54–56); therefore, the 95%
confidence intervals (CIs) are wider than they would be if each point was based on all aspirin trials irrespective of dose. Nevertheless, Fig. 1 illustrates that the cardiovascular benefit of treatment correlates strongly with the background risk across the eight populations (Spearman correlation coefficient = .86). Approximately three additional adverse events are prevented for every 10% increase in annual background risk. The largest potential benefit, not shown in Fig. 1, occurs during the month immediately following an acute MI, wherein 1 month of aspirin treatment prevents approximately 37 vascular events (MI, stroke, or death from all causes) per 1000 persons treated (50,52). Data from patients with acute MI are not included in Fig. 1 because the 1-month benefit cannot be expressed appropriately in annual terms. The cardiovascular benefit of aspirin prophylaxis is smallest in low-risk populations, such as healthy male doctors, in which only 0.09 events are prevented per 1000 patient-months of treatment (56).

The principal adverse effect of aspirin at doses of less than or equal to 500 mg daily is a small increase in the risk of serious bleeding complications. The mean frequency of hemorrhagic complications, per 1000 patient-months of treatment, is 0.07 (95% CI = 0.04 to 0.09). The maximum frequencies are 0.64 and 0.65 in patients undergoing valve surgery and in those with acute MI, respectively. Within each population, the risk of serious bleeding varies with age and comorbidity (57). Serious bleeding complications are sufficiently rare that the risk is offset by potential cardiovascular benefits among patients whose background cardiovascular risk exceeds 3% annually. The risk–benefit balance is equivocal among intermediate-risk patients (i.e., those with a 1%–3% annual incidence of adverse cardiovascular events) and unfavorable among low-risk populations (i.e., those with an annual incidence of <1%). Clinicians should be aware that the calibration of risks and benefits associated with aspirin prophylaxis remains imprecise for any particular patient profile and that prudent treatment decisions require some margin for uncertainty. While aspirin prophylaxis has become standard therapy for patients with diverse high-risk cardiovascular conditions, it has not yet been proven to provide a net clinical benefit in intermediate- or average-risk settings (52).

**General mechanism of action of NSAIDs.** The mechanism of action that defines NSAIDs as a class is their ability to inhibit the COX activity of the enzyme prostaglandin G/H-synthase and thereby block the biosynthesis of prostaglandins (58). NSAIDs prevent the formation of prostaglandin H₂, the first committed step in the metabolism of arachidonic acid into a complex cascade of signaling lipids, such as prostaglandin D₂, prostaglandin E₂, prostaglandin F₂α, prostaglandin I₂, and thromboxane, the principal prostanoïd metabolite in platelets (Fig. 2). Therapeutic concentrations of NSAIDs (usually in the low micromolar range) are not known to influence other pathways of arachidonic acid metabolism except indirectly by increasing the intracellular concentration of free arachidonic acid, which potentially causes shunting of arachidonic acid through other metabolic pathways (Fig. 2).

Two distinct isoforms of prostaglandin G/H-synthase, designated COX-1 and COX-2, have been recognized since 1991 (59,60). COX-1 is expressed constitutively in many tissues, and it plays a central role in platelet aggregation and gastric cytoprotection (61,62). Although COX-2 is expressed constitutively in the human kidney and brain, its expression is induced in many tissues during inflammation, wound healing, and neoplasia. COX-1 and COX-2 initiate the formation of biologically important prostanoïds that coordinate signaling between the cell of origin (autocrine) and neighboring cells (paracrine) by binding to transmembrane G-protein-coupled receptors (61).

NSAIDs vary in their abilities to inhibit COX-1 or COX-2 at different concentrations and in different tissues. For example, aspirin is a relatively selective inhibitor of COX-1 in platelets when given at doses of 50–100 mg daily (64,65) but inhibits COX-2 only at plasma concentrations higher than 0.5 mM. Most

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**Fig. 2.** Arachidonic acid metabolism. The major metabolites of arachidonic acid produced by the cyclooxygenase (COX) and lipooxygenase (LO) pathways are indicated. Examples of tissues in which individual prostanoids exert prominent effects are indicated in parentheses. PGD₂ = prostaglandin D₂; PGE₁ = prostaglandin E₁; PGE₂ = prostaglandin E₂; PGF₂α = prostaglandin F₂α; TXA₃ = thromboxane A₂; PGI₂ = prostaglandin I₂; HETE = hydroxyeicosatetraenonic acid; HETE = hydroxyeicosatetraenonic acid.
other conventional NSAIDs, such as ibuprofen, sulindac, and indomethacin, inhibit COX-1 and COX-2 to the same extent, whereas a new class of NSAIDs, designated coxibs by the World Health Organization, selectively inhibits COX-2 (66,67). Coxibs were developed to suppress prostanoid formation by COX-2 in inflammation while sparing the protective effects of COX-1 and its products prostaglandin H2 and prostaglandin E2 on gastric epithelium. Nevertheless, the availability of coxibs has stimulated research on the role of COX-2 in neoplasia and the potential efficacy and safety of selective COX-2 inhibition against cancer.

The pharmacologic effects of NSAIDs are further complicated by the diverse functions of prostanoids in different tissues and by the variable effects of COX inhibition, depending on drug, dose, and clinical context. The formation of specific prostanoids varies across different tissues because of differences in the concentration of tissue-specific isomerases that catalyze their production from prostaglandin H2. Furthermore, more than one G-coupled protein receptor may transduce different effects from the same prostanoid (63). The diverse effects of COX inhibition can also be either therapeutic or deleterious, depending on the clinical characteristics of the patient. For example, low-dose aspirin (30–150 mg daily) selectively inhibits the production of thromboxane A2 in platelets by COX-1, thereby suppressing platelet aggregation, vasoconstriction, and hemostasis (64,65). This antithrombotic effect may be beneficial for the majority of patients at high risk of occlusive vascular disease but deleterious for those who develop bleeding complications. Anti-inflammatory doses of NSAIDs (e.g., ibuprofen at a dose of 800 mg every 8 hours or naproxen at a dose of 500 mg twice daily) are therapeutic for patients with osteoarthritis or rheumatoid arthritis but can cause gastrointestinal ulceration, bleeding, or disruption of renal hemodynamics in susceptible patients. A current controversy concerns whether long-term use of coxibs, which minimize serious gastrointestinal toxicity, may promote thrombosis or offset the cardiovascular benefits of low doses of aspirin by suppressing prostacyclin in vascular endothelial cells (63). Although coxibs do decrease urinary excretion of prostacyclin metabolites in normal subjects (69–71), currently available data do not resolve whether prolonged suppression of COX-2 may adversely affect thrombosis. Given the biologic complexity of prostanoid metabolism, however, it is not surprising that drugs that inhibit the activity of COX isoenzymes can have untoward as well as desirable effects on human health.

**Strategies to improve the selectivity of NSAIDs.** Several generic strategies have been developed to improve the selectivity and reduce the toxicity of NSAIDs. One strategy is to identify high-risk populations in which the benefits of treatment would outweigh any attendant toxicity. A second strategy, which is taken by pharmaceutical companies, is to develop novel coxibs that inhibit COX-2 and suppress inflammation in chronic arthritis patients while sparing COX-1, thus avoiding the most serious gastrointestinal toxic effects (67,72). Currently available drugs with these properties are celecoxib and rofecoxib. Other highly selective COX-2 inhibitors, such as valdecoxib, etoricoxib, and COX-189, are now completing phase III trials of efficacy and safety in patients with osteoarthritis and rheumatoid arthritis.

A third approach to improve the selectivity and reduce the toxicity of NSAIDs is to determine the lowest effective drug dose and the most critical period for administration to achieve a specific pharmacologic effect. For example, low-dose aspirin (50–100 mg daily) is as effective as aspirin at higher doses in inhibiting COX-1 activity in platelets (64,65) and preventing MI and thrombotic stroke (52) while minimizing gastrointestinal toxicity. Decisions about the optimal treatment regimen and lowest effective dose are more easily made when the mechanism of action is known. For example, aspirin is the only NSAID that covalently and irreversibly inactivates COX-1, and it does this at concentrations that reach platelets in the enterohepatic circulation but are largely metabolized by the liver before entering the systemic circulation (53). Platelets lack a nucleus and, therefore, cannot synthesize new COX-1 during their 7- to 10-day life span. This combination of factors allows once-daily use of low-dose aspirin to provide full protection against platelet aggregation, despite its half-life of approximately 20 minutes in the systemic circulation (53).

A fourth strategy to improve the balance of benefits and risks associated with NSAID use is to identify combinations of drugs that are effective at very low doses (73,74). For example, much lower doses of sulindac and the cholesterol-lowering drug lovastatin are required to suppress chemically induced cancer in rodents and to stimulate apoptosis in human tumor cells when the drugs are given simultaneously than when either drug is given alone (75). Besides lovastatin, a 3-hydroxy-3-methylglutaryl coenzymeA reductase inhibitor, other drugs that have also been used in combination with either sulindac (73), aspirin (76), or piroxicam (77,78) include ornithine decarboxylase inhibitors (76–78), the spice curcumin (79), and EKI-785, an irreversible inhibitor of the epidermal growth factor receptor kinase (73).

**Evidence for Cancer Prevention Properties of NSAIDs**

Chemically induced intestinal cancer in rodents. The hypothesis that NSAIDs might inhibit the occurrence or growth of colorectal cancer arose in the mid-1970s, when Bennett and Del Tacca (80) and Jaffe (81) reported that the concentration of prostaglandin E2 was higher in human colorectal tumor tissue than in the surrounding normal mucosa. These relatively crude measurements of prostaglandin concentrations in human tumors stimulated more than 40 experiments in which numerous NSAIDs were shown to inhibit chemically induced colorectal cancer or aberrant crypt formation in rats or mice (76–79,82–116). In these animal models, weanling rats or mice were given a subcutaneous injection of azoxymethane or other carcinogens known to induce intestinal cancer and were subsequently given known concentrations of NSAIDs in their food or water. Those experiments varied the timing of the NSAID treatment in relation to the carcinogen exposure by initiating treatment before exposure to the carcinogen (initiation phase), during the promotion-progression phase, or both. Colorectal tumors produced in the rat model share many characteristics with human colorectal cancer, except the former have a lower tendency to metastasize (117).

The studies in rodents proved conclusively that aspirin, other conventional NSAIDs (such as piroxicam, indomethacin, sulindac, ibuprofen, and ketoprofen), and selective COX-2 inhibitors [e.g., celecoxib (107)] inhibit chemically induced carcinogenesis in rats and mice (46). The highest tolerated dose of nonselective NSAIDs typically reduced the number and size of tumors by 40%–60%. Nonselective NSAIDs suppressed but did not completely eliminate the growth of chemically induced adenomatous polyps and cancers. Two studies of coxibs in this model
(107,114) have indicated that high doses of celecoxib (1500 ppm in food) inhibit 90% of tumors in rats and are better tolerated than comparable doses of nonselective NSAIDs.

At least three important insights can be derived from the rodent experiments. First, nonselective NSAIDs suppress tumor growth to a greater extent and at lower doses when treatment is begun before or coincident with exposure to the carcinogen than when it is delayed until the tumor promotion/progression phase. For example, low-dose piroxicam (25 ppm in food) caused a 30% reduction in tumors when treatment was begun soon after exposure to the carcinogen but only a 12% reduction when treatment was begun 23 weeks after exposure (98). Early initiation of treatment also improves tumor suppression by sulindac sulfone (110) and celecoxib (118). Second, both nonselective and selective NSAIDs effectively inhibit the early stages of tumor development, whereas only selective COX-2 inhibitors are effective when treatment is delayed. For example, celecoxib (1500 ppm in food) reduced tumor incidence and multiplicity by approximately half, even when treatment was delayed until the tumor promotion/progression stage (118). Third, NSAID treatment must be continued without interruption to prevent resumption of tumor growth (91–92).

Experimental studies in rodents have several advantages not offered by other types of studies. First, the ability to administer measured concentrations of single NSAIDs to intact animals provides a greater opportunity to examine complex interactions within and across cells than exists in studies of isolated cell cultures and other in vitro models. Second, the rodent experiments are not subject to confounding by lifestyle factors, which may afflict epidemiologic studies. Limitations of the rodent experiments, other than potential interspecies differences, include a lack of uniformity in experimental design that limits comparisons across studies and the lack of measurements of NSAID concentrations in blood, COX-1 activity in platelets, or COX-2 activity in activated monocytes. Measurements of COX-2 activity in activated monocytes could help to identify the enzymatic target involved in tumor inhibition.

Other experimental studies indicate that NSAIDs inhibit many induced and transplanted cancers in various animal models, although the evidence for this is more limited than that for colorectal cancer. Other cancers potentially affected by NSAIDs include tumors of the esophagus (119–121), stomach (122,123), skin (124), breast (125–128), lung (129–132), prostate (133,134), and urinary bladder (135–137). A comprehensive review of these studies is beyond the scope of this review.

**Clinical studies and randomized trials of NSAIDs in FAP.** Randomized clinical trials have established that two NSAIDs, sulindac (41,42) and celecoxib (44), suppress adenomatous polyps and cause regression of existing polyps in patients with FAP (Table 1). FAP is a rare hereditary condition resulting from germline inactivation of one allele of the adenomatous polyposis coli (APC) gene. Affected individuals develop tens to thousands of adenomatous polyps. If these individuals do not undergo surgical resection of the colon, virtually all develop colorectal cancer by the third or fourth decade of life (138). FAP accounts for only 1% of human colorectal cancers, yet it provides a model of APC inactivation as an early genetic event for the approximately 85% of cancers that develop from sporadic adenomatous polyps. Treatment with both sulindac and celecoxib is used to supplement surgery in FAP patients (139). However, it should be noted that some FAP patients have developed rectal carcinoma, despite ongoing therapy with sulindac (140,141) and that adenomatous polyps resume growth in FAP patients if NSAID prophylaxis is stopped. The one published study that evaluated NSAID prophylaxis in relation to the regression of small (<1 cm) sporadic adenomatous polyps (142) found no statistically significant difference in polyp size among the 18 patients treated with sulindac (300 mg) for 4 months.

**Mouse models of FAP.** Several murine models that resemble human FAP have been developed and used to determine whether various NSAIDs and coxibs suppress the development of spontaneous intestinal adenomas (143). Nonselective NSAIDs, such as piroxicam (144), sulindac (145–147), and aspirin (148), and selective COX-2 inhibitors, such as celecoxib (144) and rofe-

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**Table 1. Published randomized clinical trials of NSAIDs and adenomatous colorectal polyps**

<table>
<thead>
<tr>
<th>Investigator(s), y, (reference No.)</th>
<th>Study population (total No.)</th>
<th>Drug (dose), duration</th>
<th>Phase</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steinbach et al., 2000 (44)</td>
<td>FAP (77)</td>
<td>Celecoxib (100 mg bid or 400 mg bid), 6 mos</td>
<td>II</td>
<td>Celecoxib significantly decreases the No. of colon polyps</td>
</tr>
<tr>
<td>Labayle et al., 1991 (41)</td>
<td>FAP (10)</td>
<td>Sulindac (100 mg tid), 4 mos</td>
<td>III</td>
<td>Polyps regressed completely in 6 patients, partly in 3</td>
</tr>
<tr>
<td>Giardiello et al., 1993 (42)</td>
<td>FAP (22)</td>
<td>Sulindac (150 mg bid), 9 mos</td>
<td>III</td>
<td>Sulindac decreased No. of polyps by 56% and size by 65%</td>
</tr>
<tr>
<td>Nugent et al., 1993 (43)</td>
<td>FAP (24)</td>
<td>Sulindac (400 mg), 6 mos</td>
<td>III</td>
<td>Duodenal polyps &lt;2 mm regressed in 9 of 11 patients treated with sulindac</td>
</tr>
</tbody>
</table>

| Fu et al., 1994 (143)              | Previous adenaomatous polyps (64) | Sulindac (300 mg), 4 mos | III   | Sulindac did not statistically significantly decrease No. or size of polyps |
| Ruffin et al., 1997 (203)          | High risk of colorectal cancer: FAP, HNPPC (65) | Aspirin (40, 80, 160, 320, or 648 mg qd), 2 wk | I     | Lowest effective dose of aspirin to prevent cancer may be 81 mg daily |
| Carbene et al., 1998 (204)         | Dukes’ A colon cancer or other conditions (40) | Piroxicam (10 mg qd or 10 mg qod) and DFMO, 6 mos | I     | Small doses of DFMO and piroxicam may be additive in their chemopreventive effect |
| Calaluce et al., 2000 (205)        | Previous adenaomatous polyps (96) | Piroxicam (7.5 mg), 2 y | IIB   | Toxicity of piroxicam treatment may outweigh its benefit |
| Chow et al., 2000 (206)            | Previous adenaomatous polyps (27) | Ibuprofen (300 mg qd or 600 mg qd), 4 wk | I     | Lowest effective dose of ibuprofen to prevent cancer may be 300 mg daily |

*NSAIDs = nonsteroidal anti-inflammatory drugs; FAP = familial adenomatous polyposis; bid = twice a day; tid = three times a day; HNPPC = hereditary nonpolyposis colon cancer; qd = every day; qod = every other day; DFMO = α-difluoromethylornithine.

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coxib (149), inhibit tumor development in ApcMin mice and other murine models of FAP. These models mimic the rapid development of adenomatous polyps that affects humans with germline inactivation of one APC gene but differ from FAP in that the mouse tumors occur predominantly in the small intestine.

Observational epidemiologic studies in the general population. Numerous (nonrandomized) epidemiologic studies have reported that people who regularly use aspirin and other NSAIDs have a lower incidence of adenomatous polyps and lower incidences of or deaths from colorectal cancer compared with nonusers (Fig. 3) (4–40). The consistency of these findings is striking, despite different researchers using various study designs in diverse patient populations. Sustained NSAID use is associated with a 30%–50% reduction in adenomatous polyps, incident disease, and death from colorectal cancer in all but one of these epidemiologic studies (38), as seen in Fig. 3, A.

Results from these studies strongly support the hypothesis that NSAIDs inhibit the occurrence and/or progression of colorectal cancer in the general population, not just in FAP patients (150,151). The aggregate findings cannot be attributed to earlier detection because of aspirin-induced bleeding or measured potential confounders. Besides attracting substantial research interest and funding to the NSAID hypothesis, the results of these epidemiologic studies suggest that the duration and continuity of NSAID use may be more critical than the daily dose (12,15,152). Like the rodent experiments and clinical studies of FAP, the epidemiologic studies also suggest that tumors resume growth after termination of NSAID treatment (19,30).

Epidemiologic studies cannot, however, provide randomized evidence that NSAIDs prevent the development of adenomatous polyps or cancer nor have past analyses fully defined the optimal drug, dose, treatment regimen, age to begin prophylactic therapy, or the balance of risks and benefits in different patient populations. Nevertheless, these studies raise an intriguing mechanistic question in that the dose and dosing frequency of aspirin associated with lower risk of colorectal cancer are often insufficient to sustain COX-2 inhibition in nucleated cells (6,10). The possibility that activated platelets may contribute to the induction of COX-2 is discussed below.

Other epidemiologic studies have found that prolonged use of NSAIDs is associated with lower incidence of or deaths from cancers at several other sites. The literature regarding these other cancers includes studies of tumors of the esophagus (7), stomach (7), breast (5,7,9,16,38,153–157), lung (7), prostate (7,158), urinary bladder (7), and ovary (7,159–161). However, there are fewer studies of these other cancers than of colorectal cancer and the results are less consistent.

Completed randomized trials in the general population. The Physicians’ Health Study is the only randomized clinical trial of aspirin in the primary prevention of cardiovascular endpoints of sufficient size to measure incidence or death rates from colorectal cancer, even though the aspirin arm of this trial was terminated after 5 years (56). This study showed no reduction in either invasive or in situ colorectal cancer incidence nor a reduction in colorectal cancer mortality among 22,071 male physicians who were randomly assigned to receive either 325 mg of aspirin or placebo every other day for 5 years, with a 12-year follow-up (56). However, interpretation of these results is limited because of the short duration of randomized treatment, the lack of systematic screening for adenomatous polyps or cancer at the beginning and end of the trial, and the relatively low dose of aspirin tested.

Mechanistic studies of NSAIDs and apoptosis. Despite continuing uncertainty about the molecular pathways by which NSAIDs may inhibit colorectal neoplasia, there is mounting evidence that tumor inhibition may be mediated by at least two distinct cellular processes. These involve the ability of NSAIDs to restore apoptosis in APC-deficient cells (162,163) and their capacity, particularly in the case of coxibs, to inhibit angiogenesis.

Apoptosis, or programmed cell death, is needed to maintain homeostasis in continuously replicating tissues such as the intestine (164). Partial suppression of apoptosis occurs early in tumorigenesis in approximately 85% of human colorectal cancers due to the inactivation of both alleles of the APC gene (165,166). The suppression of apoptosis allows APC-deficient cells to accumulate in adenomatous polyps. Further suppression of apoptosis occurs as these cells develop additional genetic mutations and phenotypic changes (167).

In vitro, both nonselective NSAIDs and selective COX-2 inhibitors stimulate apoptosis in APC-deficient cells that have not yet undergone malignant transformation. This is also seen clinically in FAP patients treated with sulindac (168) and in experimental studies of ApcMin mice (145,147,148,169) and rats exposed to chemical carcinogens (170). Nonselective NSAIDs lose their ability to inhibit chemically induced tumors when polyps undergo malignant transformation. In contrast, selective COX-2 inhibitors stimulate apoptosis and suppress growth in many cancers, including cultured human cancers of the stomach (171), esophagus (121,172), tongue (173), brain (174), lung (130), and pancreas (175).

The precise mechanism by which NSAIDs restore apoptosis remains controversial (176), although it clearly affects factors related to APC deficiency or the induction of COX-2 or both. Apoptosis can be suppressed in normal human or rodent intestinal epithelial cells by manipulating these cells to overexpress COX-2 (162,177). In human HT-29 colon cancer cells, apoptosis can be restored by treatment with selective (178) or nonselective (179,180) COX inhibitors or by restoring APC gene function (165). Apoptosis becomes progressively more inhibited during the development of colorectal cancer (167), coincident with the increasing expression of COX-2. For example, COX-2 is undetectable in normal epithelium but is detectable in 40% of adenomatous polyps (181) and in more than 80% of colorectal cancers. COX-2 expression in human colorectal carcinomas is associated with larger tumor size and deeper invasion, although not with metastases (182).

Other studies (183,184) suggest that COX-1 activity, perhaps through the induction of COX-2, may also be essential for the development of colorectal neoplasia. In mouse knockout studies (183,184), deletion of either the COX-1 or COX-2 genes in Apc-deficient mice caused a 70%–80% reduction in intestinal polyposis. We have hypothesized that COX-1 activity in activated platelets may signal the increased expression of COX-2 in other cells through the release of lipid or protein paracrine mediators (185). A role for COX-1 in the induction of COX-2 might explain why, in epidemiologic studies, aspirin use is associated with reduced risk of colorectal cancer even at doses and dosing intervals that could not sustain COX-2 inhibition in nucleated cells (6,12).

Despite these observations, results from other studies chal-
Fig. 3. Epidemiologic studies of the association between nonsteroidal anti-inflammatory drug (NSAID) use and colorectal cancer or adenomatous polyps. The relative risk estimates (circles) and 95% confidence intervals (lines) refer to the incidence or death rate among regular NSAID users compared with that among nonusers in A) cohort studies, B) case-control studies of NSAIDs and colorectal cancer, and C) studies of NSAIDs and adenomatous polyps.
lenges the conventional wisdom that COX inhibition is the only shared function of NSAIDs (186) or that the products rather than the substrate of COX activity mediate its biologic effects. For example, in some experimental models, the concentration of free arachidonic acid itself regulates apoptosis in colorectal epithelial cells (187,188). Other experimental models suggest that NSAIDs may affect apoptosis through a mixture of prostaglan-
dependent and prostaglandin-independent pathways (176). The selective COX-2 inhibitor NS-398 stimulates apoptosis in human S/KS colon carcinoma cells, which do not express COX-2 enzyme, as well as in HT-29 cells, which do (178). Although sulindac sulfone is believed not to inhibit COX activity, it nonetheless stimulates apoptosis in rats exposed to chemical carcinogens (110) and in human HT-29 colon carcinoma cells (189). High concentrations of sulindac sulfone and sodium salicylate reportedly modify signal transduction through either the c-MYC oncogene (190), nuclear factor-κB (191,192), or p53, a mitogen-activated protein kinase (193,194). Very high concentrations of sulindac sulfide inhibit transcriptional activation by the nuclear peroxisome proliferator-activated receptor-δ (163), a nuclear hormone receptor regulated partly by APC gene function (163,195). NSAIDs have also been reported to induce apoptosis through 15-lipoxygenase-1, independent of COX-2 (196). However, many of these effects have been demonstrated only with high concentrations of NSAIDs in vitro and are of uncertain clinical relevance.

Mechanistic studies of angiogenesis. A second cellular process by which COX-2 inhibitors may inhibit tumor growth is through inhibition of angiogenesis and neovascularization (197,198). Solid tumors must stimulate the formation of new capillary blood vessels to grow larger than approximately 2 mm in diameter (198–200). COX-2 expression is widely induced in the angiogenic vasculature of colorectal adenomatous polyps and in carcinomas of the colon, lung, breast, esophagus, and prostate (200,2001). Selective COX-2 inhibitors suppress the growth of corneal capillary blood vessels in rats exposed to basic fibroblast growth factor (201) and inhibit the growth of several human tumors transplanted into mice (201,202). Therapeutic (low micromolar) concentrations of coxibs also suppress the release of angiogenic growth factors by human or rodent colorectal cancer cells that are cocultured with vascular endothelial cells (197,198) and block migration and tube formation by the endothelial cells. In contrast, toxic concentrations of aspirin (197) or indomethacin (198) are required to block vascular endothelial tube formation. These experiments suggest that COX-2 may be essential for tumor vascularization and growth. However, the relevance of the experimental models to human colorectal cancer remains uncertain.

Implications for Ongoing and Future Scientific Research

Randomized trials of NSAIDs and prevention of colorectal cancer. Approximately 20 randomized clinical trials designed to test whether nonselective NSAIDs or coxibs inhibit the early development of colorectal cancer either have been completed (Table 1) or are ongoing (Table 2). Most are phase I and II studies to determine the bioactivity of various NSAID treatments in rectal epithelium and/or their gastric toxicity (203–206). We believe that the ongoing phase I studies could be strengthened by testing validated biomarkers of apoptosis and angiogenesis in adenomatous polyps and early-stage carcinomas. For example, quantitative measures of apoptosis in adenomatous polyps may be a more sensitive index of the biologic effect of NSAIDs than are measures of cell proliferation, aberrant crypt formation, or prostaglandin concentrations in normal rectal mucosa. Improved biomarkers are essential for phase I/II studies to identify the least toxic treatments and drug combinations for further testing.

Seven phase III trials are currently testing whether aspirin, other nonselective NSAIDs, or selective COX-2 inhibitors suppress the development of adenomatous polyps or cancer in high-risk patients (Table 2). These prevention trials will provide the first randomized evidence of the effectiveness of aspirin at doses of 80–300 mg daily or celecoxib at doses of 200–400 mg daily in inhibiting the development of sporadic adenomatous polyps in patients without FAP. Two of the studies are also testing whether aspirin and celecoxib inhibit colorectal cancer among patients with HNPCC, a condition that accounts for approximately 15% of colorectal cancers.

Measuring surrogate endpoints in high-risk populations improves the feasibility of cancer prevention studies, but it also introduces new sources of uncertainty. The prevention of adenomatous polyps is not synonymous with the prevention of colorectal cancer, nor are findings in patients with FAP or HNPCC necessarily generalizable to patients with most colorectal cancers. Furthermore, even if NSAIDs do reduce the risk of colorectal cancer, none of these trials will address the safety of administering prolonged treatment prophylactically to healthy people. However, the evaluation of appropriately validated biomarkers (164) could strengthen the ability of phase III trials to test focused mechanistic hypotheses by verifying that the intervention being tested achieves the desired pharmacologic effect under field conditions.

Trials of NSAIDs as adjuvant therapy. New trials are testing the efficacy of coxibs to treat precancerous lesions of the mouth, esophagus, and skin and as adjuvant therapy for solid tumors that express COX-2 (Table 3) (172,175,207–211). Therapeutic trials are easier to conduct and are more cost-effective than prevention trials because of their smaller size and shorter follow-up (201). Therapeutic trials can directly measure clinical endpoints, such as tumor recurrence and survival, as well as potential surrogate measures of disease. Furthermore, the high risk of cancer recurrence or progression in therapeutic settings offsets some of the constraints on toxicity that limit the prevention trials.

A strong scientific rationale supports therapeutic trials of coxibs for the treatment of cancers that express COX-2 in vascular endothelium (172,175,207–211). First, celecoxib suppresses the growth of human colon and lung tumors xenografted into rodents (212). Second, adjuvant treatment with coxibs enhances the response of human HT-29 colon cancer cells and mouse sarcoma transplants to standard chemotherapy or radiation therapy (213,214). Third, a cross-sectional clinical study has shown that COX-2 expression in human colorectal tumors is directly associated with tumor stage and size at diagnosis and is inversely associated with patient survival (215). Evidence from randomized trials suggesting that coxibs inhibit recurrence or prolong survival in early-stage colorectal cancer would stimulate further trials of adjuvant treatment of other COX-2-expressing solid tumors.

Future roles for epidemiologic studies. An important continuing role for epidemiologic studies is to quantify the benefits and risks of NSAID treatment across a broader range of treat-
reveals the complex interactions that occur between adjoining molecular pathways by which this inhibition occurs. Epithelial continue to be important for identifying the cellular targets and cancer in rats have established that numerous NSAIDs inhibit toxicities is demonstrated conclusively. Epidemiologic studies can randomized studies must generally be stopped when benefit or systematic approach. Additional mechanistic insights can be obtained by understanding of the complex intracellular signaling that occurs in intact animals but may not be replicated by cell culture or other laboratory models (217,218). Studies using transgenic animals can also identify the genetic and epigenetic effects of NSAIDs. Deletion of the genes that code for enzymes (e.g., inducible prostaglandin E2 synthase) and receptors (e.g., the thromboxane receptor) downstream of prostaglandin G/H synthase may help to characterize the lipid mediator(s) involved in the modulation of apoptosis and angiogenesis. Additional mechanistic insights can be obtained by measuring NSAID concentrations in plasma, COX-1 activity in circulating platelets, and COX-2 activity in activated monocytes.

**Table 2. Ongoing clinical trials of NSAIDs and colorectal adenomatous polyps**

<table>
<thead>
<tr>
<th>Principal investigator:</th>
<th>Endpoint</th>
<th>Study population (total No.)</th>
<th>Drug (dose), † duration</th>
<th>Phase</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin study</td>
<td>Adenomatous polyps</td>
<td>Previous adenomatous polyps (112)</td>
<td>Aspirin (81 mg qd), 12 wk</td>
<td>II</td>
<td>2000–2001</td>
</tr>
<tr>
<td>Sinicrope: M.D. Anderson-CAPT Study (DM93-129)‡</td>
<td>Adenomatous polyps, survival</td>
<td>Early/late-stage Dukes’ A and B (900)</td>
<td>Aspirin (325 mg qd), 4 y</td>
<td>III</td>
<td>1993–2003</td>
</tr>
<tr>
<td>Schilsky: U Chicago/Sandler: UNC (NCI-P93-0048)§</td>
<td>Adenomatous polyps</td>
<td>Previous adenomatous polyps (700)</td>
<td>Aspirin (80 mg qd or 325 mg qd) and/or folate, 3 y</td>
<td>III</td>
<td>1994–2001</td>
</tr>
<tr>
<td>Baron: Dartmouth (NCI-P95-0063)§</td>
<td>Adenomatous polyps</td>
<td>Previous adenomatous polyps (1000)</td>
<td>Aspirin (300 mg qd and/or folic acid (500 µg qd), 3 y</td>
<td>III</td>
<td>1997–2003</td>
</tr>
<tr>
<td>Logan: Netherlands, UK</td>
<td>Adenomatous polyps</td>
<td>FAP carriers (400)</td>
<td>Aspirin (600 mg qd) and/or resistant starch (30 g), 1 y</td>
<td>III</td>
<td>1993–na</td>
</tr>
<tr>
<td>Burn: Newcakely-on-Tyne, UK</td>
<td>Adenomatous polyps</td>
<td>HNPCC carriers (1000)</td>
<td>Aspirin (600 mg qd) and/or resistant starch (30 g), 2 y</td>
<td>III</td>
<td>1999–2001</td>
</tr>
<tr>
<td>Burn: Newcakely-on-Tyne, UK</td>
<td>Adenomatous polyps</td>
<td>Effects on colonic epithelium and mucosa, dosage</td>
<td>Average or above average risk of colon cancer (130)</td>
<td>na</td>
<td>1996–na</td>
</tr>
<tr>
<td>Other nonselective NSAIDS study</td>
<td>Adenomatous polyps</td>
<td>Effects on colon crypt and rectal epithelium</td>
<td>Previous adenomatous polyps, no FAP (240)</td>
<td>Sulindac (150 mg bid), 6 mos</td>
<td>II</td>
</tr>
<tr>
<td>Shift: Rockefeller (NCI-V98-1425)§</td>
<td>Adenomatous polyps</td>
<td>Bioactivity (COX-2 and PGE2 expression), tolerability, safety</td>
<td>HNPCC patients and gene carriers (81)</td>
<td>Celecoxib, 1 y</td>
<td>I/II</td>
</tr>
<tr>
<td>Holt: Columbia (NCI-P97-0110)§</td>
<td>Adenomatous polyps</td>
<td>Bioactivity (COX-2 and PGE2 expression), tolerability, safety</td>
<td>Previous adenomatous polyps (400)</td>
<td>Sulindac (150 mg bid), 6 mos</td>
<td>II</td>
</tr>
<tr>
<td>Meyskens: UC Irvine (NCI-P00-0150)§</td>
<td>Adenomatous polyps</td>
<td>Bioactivity (COX-2 and PGE2 expression), tolerability, safety</td>
<td>Previous adenomatous polyps, no FAP (240)</td>
<td>Sulindac (150 mg qd) and DFMO (500 mg/m² qd), 3 y</td>
<td>III</td>
</tr>
<tr>
<td>Selective COX-2 inhibitors study</td>
<td>Adenomatous polyps</td>
<td>Previous sporadic adenomatous polyps (1000)</td>
<td>HNPCC patients and gene carriers (81)</td>
<td>Celecoxib, 3 y</td>
<td>III</td>
</tr>
<tr>
<td>Bertagnolli: Brigham and Women’s Hospital (NCI-P00-0141)§</td>
<td>Adenomatous polyps</td>
<td>Bioactivity (COX-2 and PGE2 expression), tolerability, safety</td>
<td>Previous sporadic adenomatous polyps (1600)</td>
<td>Celecoxib alone and with selenium, 3–5 y</td>
<td>III</td>
</tr>
<tr>
<td>Albers: Arizona Cancer Center¶</td>
<td>Adenomatous polyps</td>
<td>Previous sporadic adenomatous polyps (2400)</td>
<td>Celecoxib alone and with selenium, 3–5 y</td>
<td>III</td>
<td>2001–2004</td>
</tr>
</tbody>
</table>

*NSAIDs = nonsteroidal anti-inflammatory drugs; PI = principal investigator; CAPT = Calcium Aspirin Prevention Trial; qd = every day; UNC = University of North Carolina; NCI = National Cancer Institute; UK = United Kingdom; CAPP1 = Concerted Action Polyposis Prevention 1; FAP = familial adenomatous polyposis; HNPCC = hereditary nonpolyposis colon cancer; CAPP2 = Concerted Action Polyposis Prevention 2; na = not available; bid = twice a day; UC = University of California; DFMO = α-difluoromethylornithine; PGE2 = prostaglandin E2.

†Information on endpoint, dose, and end of study not available for some studies.

‡Clinical Trials, The University of Texas M. D. Anderson Cancer Center (http://www.mdanderson.org/patients_public/clinical_trials/).


¶Current Controlled Trials, Medical Research Council (http://www.controlled-trials.com/)


evidence of their efficacy in populations other than those with FAP and against endpoints other than adenomatous colorectal polyps. In addition, unresolved questions about the mechanism(s) by which these drugs act, the optimal drug, dose, treatment regimen, and the balance of risks and benefits in specific populations must be answered. We hope that the issues raised by this review will help to sustain progress in this exciting area.

**REFERENCES**

(4) Mulshine: National Cancer Institute; na = not available; UAB = University of Alabama, Birmingham.

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
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<th>Study population (Total No.)</th>
<th>Drug (dose),† duration</th>
<th>Phase</th>
<th>Years</th>
</tr>
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<tbody>
<tr>
<td><strong>Selective COX-2 inhibitors study</strong></td>
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<tr>
<td>Boyle: Memorial Sloan-Kettering Cancer Center</td>
<td>Efficacy (clinical &amp; histological response), safety</td>
<td>Oral premalignant lesions (84)</td>
<td>Celecoxib, 12 wk</td>
<td>II</td>
<td>2000–2001</td>
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<tr>
<td>Forastiere: Johns Hopkins University</td>
<td>Dysplasia regression</td>
<td>Low or high grade Barrett’s esophageal dysplasia (200)</td>
<td>Celecoxib, 48–96 wk</td>
<td>II</td>
<td>2000–na</td>
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<tr>
<td>Dawsey: National Cancer Institute (Linxian, China)</td>
<td>Dysplasia regression</td>
<td>Esophageal squamous dysplasia (240-600)</td>
<td>Celecoxib and selenium, 1 y</td>
<td>II</td>
<td>1999–2000</td>
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<tr>
<td>Elmets: University of Alabama Birmingham</td>
<td>Regression/prevention of actinic keratoses, biomarkers, safety</td>
<td>Actinic keratoses (300)</td>
<td>Celecoxib, 1–2 y</td>
<td>II/III</td>
<td>2000–na</td>
</tr>
<tr>
<td>Sabichi: M.D. Anderson Cancer Center</td>
<td>Time to recurrence, biomarkers, toxicity</td>
<td>Superficial transitional cell bladder carcinoma (200)</td>
<td>Celecoxib and trastuzumab, duration na</td>
<td>II</td>
<td>2000–na</td>
</tr>
<tr>
<td>Dang: Memorial Sloan-Kettering Cancer Center</td>
<td>Efficacy, safety</td>
<td>Metastatic breast cancer (12–25)</td>
<td>Celecoxib, 6 wk</td>
<td>I</td>
<td>2001–na</td>
</tr>
<tr>
<td>Carducci: Johns Hopkins University</td>
<td>Biomarker (prostaglandin levels, toxicity)</td>
<td>Localized prostate cancer (60–70)</td>
<td></td>
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</tr>
</tbody>
</table>

*PI = principal investigator; NCI = National Cancer Institute; na = not available; UAB = University of Alabama, Birmingham.

Table 3. Therapeutic trials of selective COX-2 inhibitors and endpoints other than colorectal adenomatous polyps or cancer*


(116) Lehnter T, Deschner EE, Karmali RA, DeCosse JJ. Effect of flurbiprofen

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NOTES

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