Cancer Prevention: A New Era beyond Cyclooxygenase-2

Perspectives in Pharmacology

Basil Rigas and Khosrow Kashfi
Division of Cancer Prevention, Department of Medicine, The State University of New York at Stony Brook, Stony Brook, New York (B.R.); and Department of Physiology and Pharmacology, City University of New York Medical School, New York, New York (K.K.)

Received December 16, 2004; accepted March 31, 2005

ABSTRACT
The seminal epidemiological observation that nonsteroidal anti-inflammatory drugs (NSAIDs) prevent colon and possibly other cancers has spurred novel approaches to cancer prevention. The known inhibitory effect of NSAIDs on the eicosanoid pathway prompted studies focusing on cyclooxygenase (COX) and its products. The increased prostaglandin E2 levels and the overexpression of COX-2 in colon and many other cancers provided the rationale for clinical trials with COX-2 inhibitors for cancer prevention or treatment. Their efficacy in the prevention of sporadic colon and other cancers remains unknown; one COX-2 inhibitor has been withdrawn because of side effects, and there are concerns about whether these effects are class-specific. There is evidence to suggest that COX-2 may not be the only or ideal eicosanoid pathway target for cancer prevention. Six sets of observations support this notion: the relatively late induction of COX-2 during carcinogenesis; the finding that NSAIDs may not require inhibition of COX-2 for their effect; the modest effect of coxibs in cancer prevention; that currently available coxibs have multiple non-COX-2 effects that may account for at least some of their efficacy; the possibility that concurrent inhibition of COX-2 in non-neoplastic cells may be harmful; and the possibility that COX-2 inhibition may modulate alternative eicosanoid pathways in a way that promotes carcinogenesis. Given the limitations of COX-2-specific inhibitors and the biological evidence mentioned above, we suggest that targets other than COX-2 should be pursued as alternative or complementary approaches to cancer prevention.

Cancer, a major medical challenge during the last 100 years, has touched the life of almost every American family and absorbs an inordinate amount of health care resources. In the US, cancer has moved to first place among the causes of death, surpassing cardiovascular disease. That cancer prevention is better than treatment is almost axiomatic in medicine. In contrast to cardiovascular and infectious diseases, whose prevention has had a substantial impact on their associated morbidity and mortality, gains in cancer prevention have been limited. The main reasons for this can be found in two areas: the identification of informative biomarkers and the development of safe and effective chemopreventive agents. In this perspective, we present a brief overview of current efforts to develop effective chemopreventive drugs focused on cyclooxygenase (COX) inhibition, and, taking a rather iconoclastic approach, we suggest that a search for alternative targets for drug development may be in order.

The Clear Message of Nonsteroidal Anti-Inflammatory Drugs for Cancer Prevention and Their Limitations

1988 marked a watershed point for cancer prevention; it was then that the first epidemiological demonstration that nonsteroidal anti-inflammatory drugs (NSAIDs) prevent human colon cancer established the feasibility of human cancer chemoprevention (Kune et al., 1988). That long-term use of NSAIDs prevents an array of cancers is now firmly established. This conclusion is based on two sets of data: 1) epidemiological studies that document an association between NSAID use and cancer risk and 2) interventional clinical trials demonstrating that the administration of NSAIDs can actually prevent cancer. The epidemiological studies reported to date (Thun et al., 2002), collectively describing

ABBREVIATIONS: COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; LOX, lipoxygenase; FAP, familial adenomatous polyposis; mPGES, microsomal PGE synthase; PGI2, prostacyclin.
results on >1 million subjects, have pointed out the protective effect of NSAIDs against colon (Fig. 1), esophageal, gastric, breast (estrogen receptor-positive), and perhaps pancreatic and ovarian cancers. For colon cancer, two randomized interventional studies using polyp recurrence as a general endpoint demonstrated the preventive effect of aspirin (Baron et al., 2003; Sandler et al., 2003). The relative risks following administration of aspirin ranged between 0.59 and 0.96, depending on the specific endpoint and aspirin dose. Several aspects of this effect seem unclear at this point, but the overall significance of these landmark studies proving the general concept of chemoprevention by NSAIDs cannot be overstated.

What these studies have failed to provide are the details of the big picture: which one of the nearly 30 NSAIDs is the most effective, what is the optimal dose, and what is the ideal schedule of administration? Even if such information existed, however, it would probably be of limited or no practical value. The reason is that, although we have unassailable proof of principle for chemoprevention, current NSAIDs cannot overcome two prohibitive limitations concerning their safety and efficacy. For example, for colon cancer, the one most thoroughly studied, NSAIDs can prevent at best 50% of the cases (Thun et al., 2002). Although no precise numbers are available for the incidence of the side effects of NSAIDs, it seems that among patients using NSAIDs, up to 4% per year suffer serious gastrointestinal complications (Bjorkman, 1999). The issue of safety is clearly underscored by the report that in 1998 in the US the number of deaths from NSAID-induced gastrointestinal complications was virtually equal to the number of deaths from AIDS (Singh and Triadafilopoulos, 1999).

These considerations should be viewed against the fundamental distinction between chemotherapy and chemoprevention. In chemotherapy, both patient and physician accept substantial treatment-related toxicity to save the patient’s life from a fully developed cancer. In contrast, chemoprevention agents will be often prescribed to a healthy subject for a cancer that may never develop. Thus, for those subjects, safety and efficacy ought to assume a different value, and the overall significance of these landmark studies proving the general concept of chemoprevention by NSAIDs cannot be overstated.

What these studies have failed to provide are the details of the big picture: which one of the nearly 30 NSAIDs is the most effective, what is the optimal dose, and what is the ideal schedule of administration? Even if such information existed, however, it would probably be of limited or no practical value. The reason is that, although we have unassailable proof of principle for chemoprevention, current NSAIDs cannot overcome two prohibitive limitations concerning their safety and efficacy. For example, for colon cancer, the one most thoroughly studied, NSAIDs can prevent at best 50% of the cases (Thun et al., 2002). Although no precise numbers are available for the incidence of the side effects of NSAIDs, it seems that among patients using NSAIDs, up to 4% per year suffer serious gastrointestinal complications (Bjorkman, 1999). The issue of safety is clearly underscored by the report that in 1998 in the US the number of deaths from NSAID-induced gastrointestinal complications was virtually equal to the number of deaths from AIDS (Singh and Triadafilopoulos, 1999).

These considerations should be viewed against the fundamental distinction between chemotherapy and chemoprevention. In chemotherapy, both patient and physician accept substantial treatment-related toxicity to save the patient’s life from a fully developed cancer. In contrast, chemoprevention agents will be often prescribed to a healthy subject for a cancer that may never develop. Thus, for those subjects, safety and efficacy ought to assume a different value, and more demanding criteria than those applied to chemotherapy should be used. The ideal chemopreventive agent ought to have an efficacy approaching 100% and a safety profile that is nearly perfect. Such perfection, however, is probably unattainable, and the challenge may be simply to define what would constitute an acceptable deviation from the ideal compound. Agents with imperfect performance will be judged on the basis of their individual characteristics and against competing agents. Aspirin, for example, is widely employed for the prevention of cardiovascular thrombotic events, although its efficacy is far from perfect (e.g., it is only 28% for primary prevention), and the side effects even of the low dose of 81 mg daily are not insignificant (Kimmey, 2004).

Efficacy, safety, cost, and ease of administration will likely be the main criteria for candidate chemoprevention agents. A fifth criterion that may be employed in the future is the degree of risk of developing a given cancer. It is quite possible that genetic testing will be able to quantify risk as well as the likelihood for a given subject to respond to an agent, thus making individualized cancer prevention possible. Whether articulated or not, considerations of safety and efficacy have spurred the search for a “better NSAID”, with coxibs, selective inhibitors of COX-2, being the most notable outcome.

**COX-2 as a Target for Cancer Prevention: Pros and Cons**

The development of coxibs has been based on the notion that inhibition of COX-2, the induced isoform of COX, will diminish the proinflammatory activities of COX, whereas sparing COX-1, the constitutive isoform of COX, will diminish the gastrointestinal and perhaps other side effects of nonsteroidal anti-inflammatory drugs (Simmons et al., 2004). The observation that COX-2 is overexpressed in cancer led to the idea that coxibs could serve as excellent chemoprevention agents. The pros and cons for this concept are discussed below.

**Pros**

The best known biochemical effect of NSAIDs is inhibition (suicide or competitive) of COX, an important enzyme in the eicosanoid cascade that ultimately leads to prostaglandins (PGs) and related compounds (Simmons et al., 2004). This provided an immediate and plausible explanation of the epidemiological data on NSAIDs and cancer prevention. To substantiate this conjecture, we determined the levels of various eicosanoids in human colon cancers, showing that the most striking abnormality was elevated PGE2 levels compared with uninvolved mucosa and thereby confirming earlier findings by Bennett et al. (1987), who used somewhat crude analytical methodologies (Rigas et al., 1993). DuBois and colleagues subsequently demonstrated overexpression of COX-2 in 45% of colon adenomas and 85% of colon carcinomas (Eberhart et al., 1994). Overexpression of COX-2 has been demonstrated for several human cancers in addition to colorectal adenocarcinomas, including gastric, breast, lung, esophageal, and hepatocellular carcinomas (Turini and DuBois, 2002). Given the potentially enormous significance of this finding, a recent study assessed the possibility that single-nucleotide COX-2 polymorphisms may be associated with disease or with individual responses to drug therapies. Lin et al. (2002) reported a reduced association between the COX-2 V511A polymorphism and susceptibility to colon cancer in African Americans. Sequencing of the COX-2 gene of 72

---

**Fig. 1.** Epidemiological cohort studies of the association between NSAID use and colorectal cancer or adenomatous polyps. Circles, relative risk estimates; lines, 95% confidence intervals. Adapted from Thun et al. (2002).
individuals identified rare polymorphisms, including the V511A polymorphism, which, however, was not functionally important (Fritsche et al., 2001). Neither hypothesis—that polymorphism in the COX-2 gene could contribute to individual susceptibility to cancer or that COX-2 polymorphisms may have an impact on individual response to NSAIDs—seems to be supported. Polymorphisms in other genes of the eicosanoid pathway may account for interindividual differences in cancer susceptibility or response to NSAIDs.

In terms of mechanism, we showed that PGE2 increased colon cancer cell proliferation (Qiao et al., 1995), whereas DuBois's group showed that it suppressed apoptosis (Sheng et al., 1998). The role of eicosanoids in carcinogenesis has been further expanded by studies demonstrating that in certain cases, lipoxigenase (LOX) products may also play a role in carcinogenesis (Shureiqi and Lippman, 2001) (Fig. 2). It was therefore reasonable to conclude that inhibition of COX-2 would arrest carcinogenesis and thus 1) prevent cancer development and 2) regress cancer once developed. A series of detailed cell culture and animal studies and clinical trials on the use of coxibs in patients with familial adenomatous polyposis (FAP) and in patients with cancer culminated in one COX-2 inhibitor, celecoxib, receiving FDA approval for cancer prevention in patients with FAP.

**The Supportive Animal Studies.** Two sets of animal studies support the role of COX-2 in carcinogenesis: 1) studies using genetically modified animals and 2) studies using pharmacological inhibitors of COX-2. They are summarized below.

Although COX enzymes may not be acting simply as onco-
genases in tumor development (Simmons et al., 2004), several studies have indicated that they may be required for tumor-
genesis (Oshima et al., 1996) showed that deletion of COX-2 significantly decreased the number of intestinal tumors in Apc<sup>v716</sup> mice. Deletion of COX-1, however, also attenuated tumor formation in the same mice (Chulada et al., 2000). A stronger case was made by the studies of Hla's group, who showed that multiparous but not virgin female transgenic mice that overexpress the human COX-2 gene in the mammary glands exhibited a greatly exaggerated incidence of focal mammary gland hyperplasia, dysplasia, and transformation into metastatic tumors (Liu et al., 2001). The clear implication from these data is that enhanced COX-2 expression is sufficient to induce mammary gland tumorigenesis. Studies have also addressed whether COX-2 is critical for skin carcinogenesis. Overexpression of COX-2 in basal epidermal cells of transgenic mice (using the keratin 5 promoter), which leads to high levels of epidermal PGs, is insufficient for tumor induction but sensitizes the tissue to genotoxic carcinogens (Muller-Decker et al., 2002). In contrast, transgenic mice that overexpress COX-2 (under control of the keratin 14 promoter) in the epidermis and some other epithelia, developed tumors at a much lower frequency than did their littermate controls; tumors were induced by an initiation/promotion protocol (Bol et al., 2002). The latter results raised questions regarding the role of COX-2 in skin tumor development.

There have been numerous animal studies using specific COX-2 inhibitors that supported the concept that COX-2 inhibition both prevents and regresses tumors arising from a variety of tissues, including colon, lung, breast, pancreas, skin, and others (Dannenberg and Subbaramaiah, 2003). Perhaps the most dramatic result was reported by Reddy's group, who studied the effect of celecoxib on colon carcinogenesis in the azoxymethane rat model. Remarkably, dietary administration of celecoxib inhibited both the incidence and multiplicity of colon tumors by approximately 93 and 97%, respectively. It also suppressed the overall colon tumor burden by more than 87% (Kawamori et al., 1998).

Together, these studies (along with many more not reviewed here) provide a spectrum of evidence for the role of COX-2 in carcinogenesis, ranging from the compelling

**Fig. 2.** Overview of the eicosanoid pathway. Arachidonic acid, the substrate of three major biosynthetic pathways, is derived from diet and released from membrane phospholipids through a series of reactions requiring phospholipases, or synthesized from linoleic acid. The COX pathway produces various eicosanoids and thromboxane; the LOX pathways produce leukotrienes and hydroxyeicosatetraenoic acids; and the cytochrome P450 pathways produce epoxycyclostrostanoids, or synthesized from linoleic acid. The COX pathway produces various eicosanoids and thromboxane; the LOX pathways produce leukotrienes and hydroxyeicosatetraenoic acids; and the cytochrome P450 pathways produce epoxycyclostrostanoids (EEt), 13-HODE, 13-HODE, and 13-S-HODE, respectively; 12/15-LOX, 12/15-LOX, 12/15-LOX, and 12/15-LOX, respectively; TXA<sub>2</sub>, thromboxane A<sub>2</sub>, LT<sub>A</sub>, LT<sub>A</sub>, LT<sub>C</sub>, LT<sub>C</sub>, LT<sub>D</sub>, LT<sub>D</sub>, and LT<sub>E</sub>, leukotrienes A<sub>5</sub>, B<sub>5</sub>, C<sub>5</sub>, D<sub>5</sub>, and E<sub>5</sub>, respectively; PGE<sub>2</sub>, PGF<sub>2</sub>, PGI<sub>2</sub>, and PGF<sub>2</sub>, prostaglandins E<sub>2</sub>, F<sub>2</sub>, D<sub>2</sub>, and I<sub>2</sub> (prostaglycerin), respectively; PGE<sub>2</sub>, PGF<sub>2</sub>, PGI<sub>2</sub>, and PGF<sub>2</sub>, prostaglandins E<sub>2</sub>, F<sub>2</sub>, D<sub>2</sub>, and I<sub>2</sub> (prostaglycerin), respectively; PGE<sub>2</sub> synthesis into metastatic tumors (Liu et al., 2001). The clear implication from these data is that enhanced COX-2 expression is sufficient to induce mammary gland tumorigenesis. Studies have also addressed whether COX-2 is critical for skin carcinogenesis. Overexpression of COX-2 in basal epidermal cells of transgenic mice (using the keratin 5 promoter), which leads to high levels of epidermal PGs, is insufficient for tumor induction but sensitizes the tissue to genotoxic carcinogens (Muller-Decker et al., 2002). In contrast, transgenic mice that overexpress COX-2 (under control of the keratin 14 promoter) in the epidermis and some other epithelia, developed tumors at a much lower frequency than did their littermate controls; tumors were induced by an initiation/promotion protocol (Bol et al., 2002). The latter results raised questions regarding the role of COX-2 in skin tumor development.

There have been numerous animal studies using specific COX-2 inhibitors that supported the concept that COX-2 inhibition both prevents and regresses tumors arising from a variety of tissues, including colon, lung, breast, pancreas, skin, and others (Dannenberg and Subbaramaiah, 2003). Perhaps the most dramatic result was reported by Reddy's group, who studied the effect of celecoxib on colon carcinogenesis in the azoxymethane rat model. Remarkably, dietary administration of celecoxib inhibited both the incidence and multiplicity of colon tumors by approximately 93 and 97%, respectively. It also suppressed the overall colon tumor burden by more than 87% (Kawamori et al., 1998).

Together, these studies (along with many more not reviewed here) provide a spectrum of evidence for the role of COX-2 in carcinogenesis, ranging from the compelling
(breast carcinogenesis) to the rather weak and controversial (skin carcinogenesis). Overall, the evidence from animal studies strongly suggests that COX enzymes, particularly COX-2, participate in a significant way in carcinogenesis.

Cons

There are six sets of observations that challenge the intellectual beauty and utter simplicity of the notion that COX-2 is central to the pathogenesis of several cancers and that its inhibition would prevent them and regress those already established. They include 1) the relatively late induction of COX-2 during carcinogenesis; 2) the finding that NSAIDs, which prevent cancer, may not require inhibition of COX-2 for their effect; 3) the modest effect of coxibs in cancer prevention; 4) the fact that currently available coxibs have multiple non-COX-2 effects that may account for at least some of their efficacy; 5) the possibility that concurrent induction of COX-2 in non-neoplastic cells may be harmful; and 6) the possibility that COX-2 inhibition may modulate alternative eicosanoid pathways in a manner that promotes carcinogenesis. Below, we briefly discuss these points as well as the potential role of the large family of eicosanoids in carcinogenesis.

Pattern of COX-2 Expression. The pattern of COX-2 expression during colon carcinogenesis does not entirely fit the model that COX-2 is central to carcinogenesis. There is no COX-2 expression in human aberrant crypt foci, the earliest recognizable premalignant lesion in the colon (Nobuoka et al., 2004). For COX-2 to be the ideal chemoprevention target, its expression in aberrant crypt foci would be highly advantageous, if not a requirement, because chemoprevention would be easiest at this stage of lower complexity. Moreover, the expression of COX-2 commences only at the adenoma stage when only 45% of the adenomas are positive; of the carcinomas, 85% are positive (Eberhart et al., 1994).

This pattern of COX-2 overexpression is subject to an alternative interpretation, namely that COX-2 expression is the result of and not a dominant contributor to carcinogenesis. The strongest argument against this interpretation are the animal studies demonstrating that disruption of either the COX-1 or COX-2 gene reduces the development of tumors in mice. Interestingly, Chulada et al. (2000) proposed that since COX-1 (as well as COX-2) plays a key role in intestinal tumorigenesis, it may also be a chemotherapeutic target for NSAIDs. A counterargument to this is the recently reported study in which targeted overexpression of human microsomal PGE synthase-1 (mPGES-1) in the alveolar type II cells of transgenic mice, accompanied by highly elevated PGE₂ production (12.2-fold over control), was not sufficient to induce lung tumors (Blaine et al., 2003).

An interesting idea has been advanced by Takeda et al. (2003), who demonstrated that most COX-2-expressing cells in the polyps of Apc<sup>min</sup> mice are stromal fibroblasts in which COX-1, COX-2, and mPGES colocalized. COX-2 was induced only in polyps >1 mm in diameter, but COX-1 was found in polyps of any size. Based on these data, they proposed that COX-1 expression in the stromal cells secures the basal level of PGE₂ that can support polyp growth to ~1 mm and that simultaneous inductions of COX-2 and mPGES support the polyp expansion beyond ~1 mm by boosting the stromal PGE₂ production.

COX-Independent Effects of NSAIDs. At variance with the notion that COX-2 has a central role in carcinogenesis is the concept, originally proposed by us, that NSAIDs do not require the presence of COX-2 to prevent cancer (Hanif et al., 1996). This was based on the finding that in vitro NSAIDs display effects compatible with cancer prevention, such as inhibition of cell proliferation, induction of apoptosis, inhibition of angiogenesis, and many others, in the absence of COX-1 or -2. This initial observation is now firmly established by many studies that point to an array of molecular targets affected by NSAIDs (Shiff and Rigas, 1999). It is unclear which one(s), if any, of these is the pathway(s) mediating the chemopreventive effect of NSAIDs. Quite astonishing in this regard has been our recent observation that a modified aspirin inhibited cell proliferation, induced apoptosis, and inhibited nuclear factor-kB activation and Wnt signaling while inducing COX-2 expression without affecting COX-1 expression (Williams et al., 2003). Of note, such COX-2 was catalytically competent, as evidenced by the proportional increase in PGE₂ production.

A reservation concerning the non-COX effects of NSAIDs is that they occur only at “industrial strength” concentrations of NSAIDs (Marx, 2001). This is not, however, entirely accurate or necessarily relevant. For example, some NSAIDs have IC<sub>50</sub> values for the induction of colon cancer cell growth in the micromolar range, with probably sulindac sulfide, the active metabolite of sulindac, having the lowest (approximately 175 μM) and aspirin the highest (2.5 mM); the IC<sub>50</sub> for sulindac approaches 1 mM (Shiff et al., 1995). The corresponding IC<sub>50</sub> for some COX-2 inhibitors seems to be around 30 μM (Grosch et al., 2001). Thus, there is a clear discrepancy between cell culture results and pharmacological efficacy. These observations prompt a reminder of the limitations of the cell culture system, whose findings should be viewed for what they are: a mere indication of what may happen in the complexity of the human organism. For example, as discussed below, sulindac is more effective in the prevention of colon cancer in patients with FAP than coxibs, a complete reversal of the conclusions that might have been reached from the cell culture data. It should also be kept in mind that cell culture systems are by their very nature time-sensitive. Most studies using cell culture systems have to be terminated by 72 to 96 h; thus, high concentrations of an agent are at times necessary to detect an effect. In contrast, in vivo chemoprevention studies, comparatively lower doses of an agent are administered for much longer periods of time, and the effect of the agent may thus become apparent. The “low dose-long duration” or “high dose-short duration” balance needs to be considered so that potentially useful agents are not unjustifiably abandoned.

The Limited Clinical Efficacy of Coxibs. When the COX-2 inhibitor celecoxib was used in FAP, suppression of the neoplastic process was modest. After 6 months, FAP patients receiving 400 mg of celecoxib twice a day had a 28.0% reduction in the mean number of colorectal polyps (p = 0.003 for the comparison with placebo) and a 30.7% reduction in the polyp burden (the sum of polyp diameters) (p = 0.001), compared with reductions of 4.5 and 4.9%, respectively, in the placebo group (Steinbach et al., 2000). Rofecoxib had a statistically significant but marginal effect on the number of polyps in FAP patients (6.8% reduction from baseline values) (Higuchi et al., 2003). Another study assessed the effect of

Downloaded from jpet.aspetjournals.org at ASPET Journals on July 10, 2017
celecoxib on duodenal polyps in FAP patients (Phillips et al., 2002). There was a trend to improvement in duodenal disease when absolute adenoma numbers were assessed, but this was not statistically significant. However, when taking a global overview of the state of the duodenum, overall, patients taking celecoxib 400 mg twice daily for 6 months had a 14.5% reduction in involved areas compared with a 14.4% for placebo ($p = 0.436$). However, patients with clinically significant disease at baseline (greater than 5% covered by polyps) showed a 31% reduction in involved areas with celecoxib 400 mg twice daily compared with 8% on placebo ($p = 0.049$).

These results could be interpreted in one of two ways: either COX-2 is not central to the neoplastic process or celecoxib is not a sufficiently strong COX-2 inhibitor. However, all available clinical and preclinical evidence suggests that it is a strong COX-2 inhibitor (Dannenberg and Subbaramaiah, 2003). Of interest, the dose required to inhibit COX-2 activity by 80% also causes a 60% inhibition in COX-1 activity (Warner et al., 1999).

Sulindac, an NSAID, had a pronounced effect on colorectal polyps in FAP patients. A statistically significant decrease in the mean number of polyps and their mean diameter occurred in patients treated with sulindac (150 mg orally twice a day) compared with those given placebo. When treatment was stopped at 9 months, compared with baseline values, the number of polyps had decreased by 56% and the diameter of the polyps by 65% (Giardello et al., 1993). Furthermore, Winde et al. (1995) performed a pilot study using rectally applied sulindac in colectomized FAP patients. All patients responded to therapy within 6 to 24 weeks; 60 and 87% of patients achieved complete adenoma reversion after 12 months at 53 and 67 mg of sulindac per day per patient on average, respectively. Reversion was evident compared with the control group. Tissue PGE$_2$ levels were greatly reduced. It has also been shown that long-term use of sulindac is effective in reducing polyp number and preventing recurrence of higher-grade adenomas in the retained rectal segment of most FAP patients (Winde et al., 1997; Cruz-Correa et al., 2002). Although the coxib and sulindac studies are not directly comparable, given the different duration of drug administration, it seems that sulindac may have a stronger effect than coxibs, at least compared with rofecoxib. These findings suggest that inhibition of targets other than or in addition to COX-2 may contribute to sulindac’s stronger chemopreventive effect in the colon.

Another disappointing result, although not directly related to chemoprevention, was the recently reported failure of celecoxib combined with trastuzumab to have an effect on patients with human epidermal growth factor receptor-2/neu-overexpressing, trastuzumab-refractory metastatic breast cancer (Dang et al., 2004). Preclinical studies had demonstrated a link between overexpression of human epidermal growth factor receptor-2/neu and COX-2 activity. Similarly, in a phase II study of metastatic colon cancer, rofecoxib in combination with chemotherapy showed increased toxicity and no efficacy (Becerra et al., 2003).

**COX-2-Independent Effects of Coxibs.** There have been extensive data establishing that coxibs have several COX-2 independent activities that may account for at least part of their cancer-preventive properties (Table 1). Relevant to this argument are studies showing that, for example, celecoxib inhibits the growth of various cancer cell lines (Maier et al., 2004), including hematopoietic cell lines (Wasikewich et al., 2002) that are COX-2-deficient. Moreover, celecoxib inhibited the growth of COX-2-deficient colon cancer xenografts in nude mice, providing strong evidence for its COX-2-independent antitumor effect (Grosch et al., 2001). Quite recent data suggest a disturbing COX-2-independent effect of selective COX-2 inhibitors. Whereas in COX-2-positive pancreatic cancer a selective COX-2 inhibitor reduced tumor growth and angiogenesis, the opposite effect was observed in COX-2-negative pancreatic cancer. That is to say, the selective COX-2 inhibitor increased angiogenesis and tumor growth (Eibl et al., 2005).

These findings allow an alternative interpretation of the existing data, one that is at variance with the idea that COX-2 is a preeminent, if not altogether the target for cancer prevention. Thus, the chemopreventive effect of COX-2-specific inhibitors may be due to their effect on these targets and not on COX-2. If this reasoning is correct (and it may not be), it may reflect the classic limitation encountered when evaluating biology using pharmacological inhibitors: additional effects by the inhibitor can never be ruled out, making conclusions always subject to reinterpretation.

A few years ago, we proposed a model that attempted to resolve the apparent contradiction between data suggesting that both COX-2 and non-COX-2 inhibition were preventing cancer (Rigas and Shiff, 2000). Analysis of the then-available data led us to suggest that inhibition of either the COX-2 pathway alone or a non-COX-2 pathway(s) alone could prevent cancer. We are still missing some of the information needed to validate or reject this model, mainly results of trials evaluating the efficacy of COX-2 inhibitors and their effects on relevant molecular targets. The limited efficacy of COX-2 inhibitors in FAP and the in vitro evidence that COX-2 inhibitors act also beyond COX-2 inhibition suggest that the first part of our conclusion, i.e., that COX-2 inhibition alone is sufficient to arrest carcinogenesis, may be wrong. In fact, it would not be altogether surprising if it is COX-2 that is the “collateral target” in cancer prevention.

**Non-Neoplastic COX-2 Expression.** The expression of COX-2 is not restricted to tumor cells. Although COX-2 is undetectable in most tissues in the absence of stimulation, it is induced in a limited repertoire of cells, notably in monocytes, macrophages, neutrophils, and endothelial cells (Simmons et al., 2004). Among the stimulants of COX-2 induction are bacterial lipopolysaccharides, growth factors, cytokines, and phorbol esters. Relevant to the cardiovascular side effects of coxibs may be the observations that COX-2, although detectable at only low levels in vascular endothelium, may be expressed in response to normal blood flow (Topper et al., 1996). A study in healthy humans after the administration of celecoxib or ibuprofen suggests that COX-2 is a major source

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of cell proliferation</td>
<td>Badawi et al. (2004)</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>Ding et al. (2005)</td>
</tr>
<tr>
<td>Induction of cell cycle block</td>
<td>Maier et al. (2004)</td>
</tr>
<tr>
<td>Survivin expression</td>
<td>Teh et al. (2004)</td>
</tr>
<tr>
<td>Cytochrome c release</td>
<td>Sun et al. (2002)</td>
</tr>
<tr>
<td>NAG-1 induction</td>
<td>Yamaguchi et al. (2004)</td>
</tr>
<tr>
<td>Carbonic anhydrase inhibition</td>
<td>Weber et al. (2004)</td>
</tr>
</tbody>
</table>

NAG-1, NSAID-activated gene 1.
of systemic prostacyclin (PGL$_2$) biosynthesis (McAdam et al., 1999). High levels of COX-2 are detected in activated and proliferating vascular tissues, such as angiogenic microvessels and atherosclerotic tissues. Atheromatous lesions contain both COX-1 and COX-2, colocalizing mainly with macrophages of the shoulder region and lipid core periphery, whereas smooth muscle cells show lower levels (Schonbeck et al., 1999).

There is an interesting corollary to these observations that may impact the design of pharmaceutical strategies aimed at this extensive cascade of enzyme-catalyzed reactions. Eicosanoids often form “opposing pairs” whose balance determines the final result. A classic pair consists of thromboxane A$_2$ and prostacyclin, which have opposite effects on platelets and vascular tone (Simmons et al., 2004). Shifting the balance of such a pair could have either beneficial (e.g., prevention of cardiovascular events by low-dose aspirin) or catastrophic effects. The latter possibility, discussed below, may account for the cardiovascular side effects of coxibs. This may be particularly relevant to chemoprevention, in which a chemopreventive agent against cancer will be administered on a long-term basis to older subjects, i.e., those likely to have atheromatous lesions.

**COX-2 Inhibition May Modulate Alternative Eicosanoid Pathways.** The concept of the role of COX-2 in cancer should be viewed against the accumulating appreciation of the role of the eicosanoid pathways in carcinogenesis. An intricate system of talented enzymes, including phospholipases, cytochrome P450, COX, LOXs, and the so-called “terminal enzymes”, i.e., those converting endoperoxides to end products, generates an array of biologically active eicosanoids from polyunsaturated fatty acids such as arachidonic and linoleic acids (Fig. 2). At times, these eicosanoids have antithetic functions. Besides products of COX isoforms, LOX products may be important in carcinogenesis. Some LOX products have protumorigenic activities, whereas others are antitumorigenic (Shureiqi and Lippman, 2001). Some of the terminal enzymes may have their own distinctive role in cancer. For example, increased pulmonary production of PGL$_2$ by lung-specific overexpression of prostacyclin synthase, which operates downstream of COX, decreases lung tumor incidence and multiplicity in both chemically induced murine lung cancer models and in a tobacco smoke exposure model (Keith et al., 2002, 2004). Such findings suggest the possibility that COX inhibition may not be always desirable for cancer control.

Inhibition of COX may shift its substrate fatty acid to a non-COX pathway and generate a procarcinogenic end product. There is little evidence for substrate channeling from the COX-active site to the peroxidase-active site of the COX monomer. Functional coupling between COXs and phospholipase A$_2$s, however, has been described previously (Murakami et al., 2002), as has colocalization of COX isoforms with other enzymes of the eicosanoid cascade (e.g., Liou et al., 2001). Thus, a case can be considered where inhibition of COX-2 could shift arachidonic acid to the LOX pathway, thereby suppressing apoptosis—not a desirable effect in cancer prevention. A recent human study suggested that oral celecoxib increased leukotriene B$_4$ production in the lung microenvironment under physiologic conditions, although the functional significance of this effect was uncertain (Mao et al., 2004).

These seemingly subtle issues emphasize the complexity of the system and suggest the need for caution in devising chemopreventive strategies. Viewed more creatively, this situation may also represent a pharmacological opportunity; indeed, multipathway inhibition may be the next wave in chemoprevention. Such considerations have led to the development of dual inhibitors of both COX and 5-LOX. Licofelone, a compound blocking both a LOX and COX is already in clinical trials.

It is apparent from these considerations that the discovery of COX-2 overexpression in various cancers has provided a strong stimulus in the last decade to unravel many pathways likely related to cancer pathogenesis and sharpen the focus on cancer prevention as a realistic option. However, the central concept of a dominant role of COX-2 in cancer prevention may have significant limitations that necessitate its reappraisal. In terms of practical applications, its Achilles heel may be the fact that COX-2 expression is not cancer-restricted, and thus, long-term inhibition of COX-2 for cancer control may be associated with limiting or even unacceptable side effects. It is conceivable that cancer prevention achieved by current coxibs is to some extent due to their non-COX-2 effects, whereas their cardiovascular toxicity is due to their COX-2 effects. This notion could provide the basis for an alternative look at the wealth of available data.

### The Withdrawal of Rofecoxib and Its Implications for Cancer Prevention

In some patients, NSAIDs inhibit growth of colorectal adenomas. Hence, the Adenomatous Polyposis Prevention on Vioxx study was launched to evaluate the efficacy of rofecoxib in this group of patients; 2600 subjects with previously removed colorectal polyps were randomly assigned to receive rofecoxib or placebo. The data showed that 3.5% of rofecoxib recipients and 1.9% of placebo recipients suffered myocardial infarctions or strokes during the trial. This prompted the termination of this and all related trials and the permanent withdrawal of rofecoxib. This has been a setback to the use of COX-2 inhibitors to prevent colon and perhaps other cancers. At this time, it is still unclear whether or not the COX-2 inhibitor celecoxib shares this side effect and is still marketed. Although the Celecoxib Long Term Arthritis Safety Study trial showed this agent to have fewer gastrointestinal side effects and no increase in cardiovascular risk at 6 months, the 12-month data suggest that celecoxib did not differ from the traditional NSAIDs in its gastrointestinal effects, and, importantly, a retrospective analysis of the data suggests signs of increased cardiovascular risk (Silverstein et al., 2000). This has prompted concerns for a “class (side) effect” (Fitzgerald, 2004). The mechanism of the increased cardiovascular risk is not yet clear. It has been suggested that since COX-2 is the principal enzyme involved in the production of PGL$_2$, its inhibition by COX-2 inhibitors could increase cardiovascular risk by tipping the balance toward platelet aggregation and vasoconstriction (Fitzgerald, 2004). Of note, an elegant recent study showed that COX-2 derived prostacyclin confers atheroprotection on female mice, suggesting that chronic treatment of patients with selective inhibitors of COX-2 could undermine protection from cardiovascular disease in premenopausal females (Egan et al., 2004).
Two important considerations merit mention here. First, strictly speaking, the COX-2-specific inhibitors may be only COX-2-preferential inhibitors (Vane and Warner, 2000). Second, individual variations between COX-2 inhibitors may be due to their varied effect on molecular targets outside COX-2. Parenthetically, this may account for the presumed differential behavior of celecoxib compared with, for example, rofecoxib. Indeed, as mentioned earlier, these agents do have COX-2-independent activities that may account for their cancer-preventive properties.

**Targets beyond COX-2: The Rationale and the Promise**

That NSAIDs and COX-2-specific inhibitors alike modulate targets other than COX-2 is now beyond doubt and should be factored in when analyzing their effects on cancer prevention. The data discussed above make it unlikely that inhibition of COX-2 alone could prevent cancer; even COX-2-specific inhibitors may require the contribution of their non-COX-2 effects for their (probably suboptimal) effect on cancer prevention. Combined with the recent withdrawal of one COX-2 inhibitor, these data make a strong case for an evaluation of drug development targets beyond COX-2. Of note, Niitsu and his group in Sapporo have actively pursued the idea that modulating targets other than COX-2 can prevent cancer. In a series of elegant studies, they have shown that the phase II enzyme glutathione S-transferase P1-1 may be an appropriate target for chemoprevention (Niitsu et al., 2004; Nobuoka et al., 2004).

Whenever compounds, such as the various NSAIDs, modulate multiple pathways, it is crucial to assess the mechanistic relevance of each pathway. This is more so in the case of cancer that represents a process rather than an abrupt transition from normalcy to malignancy. In this context, and examined from the viewpoint of the drug, it is important to resolve the issues of dominance versus cooperation versus redundancy of pathways. It is conceivable that only one of the pathways affected by the drug may actually mediate the effect under study (dominance), the remaining being either minor accessories or simple downstream effects. In case of cooperation, concerted modulation of more than one pathway is needed to achieve the result, whereas in case of redundancy each of several pathways could autonomously arrest the process. Redundancy is of particular interest when dealing with cancer, where several subclones, each with different biochemical profiles, can develop "under pressure," mainly from the host or from treatment. An agent displaying mechanistic redundancy could affect multiple subclones and is thus better equipped to arrest or abort the carcinogenic process.

This line of reasoning underscores the need to explore targets beyond COX-2, especially in view of emerging limitations of the COX-2 approach. Developing a rational approach to cancer prevention would likely require a strategy capitalizing on two facts: 1) NSAIDs do prevent cancer in humans, and 2) NSAIDs act on multiple molecular targets, of which COX-2 is but one. With this as a point of departure, it is clear that the study of the non-COX-2 targets is likely to point to opportunities for cancer prevention hitherto unappreciated. Inherent in this approach is the need to resolve issues of mechanistic dominance, cooperation, and redundancy and then make informed choices for drug development. This represents the promise of "molecular targets beyond COX-2."


