

Review

Non-steroidal Anti-inflammatory Drugs in Cancer Prevention and Therapy

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Abstract. Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) can be regarded as an effective approach for cancer chemoprevention, as demonstrated by a bulk of clinical and experimental evidence. However, the clinical use of these drugs as chemopreventive agents is limited by many open questions about the optimal drug, dose, duration of therapy and knowledge about the mechanism(s) by which these drugs act. In particular, the recent data on cardiovascular toxicity of coxibs has posed some limitations on the use of NSAIDs for cancer chemoprevention in the general population. The situation is different in certain genetically susceptible subgroups, such as in individuals with genetic mutations associated with hereditary nonpolyposis colon cancer (HNPCC) or familial adenomatous polypos (FAP) in whom lifetime risk increases up to 70-90% and in whom the benefit of a chemopreventive drug might justify its use even in the presence of adverse effects.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, and ibuprofen are widely used as effective anti-inflammatory, antipyretic and analgesic drugs, and aspirin is also effective in both the primary and secondary prevention of cardiovascular diseases. Since the first epidemiological demonstration in 1988 that NSAIDs prevent human colon cancer (1), evidence has been accumulating suggesting that long-term use of NSAIDs can be regarded as

an effective approach for cancer chemoprevention (2). In contrast to cardiovascular disease, whose prevention has had a substantial impact on its associated morbidity and mortality, improvements in cancer prevention have been limited. The main reasons for this can be found in the limitations encountered in the search for safe and effective chemopreventive agents in average- or low-risk populations (3). One limitation, for example, is given by 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 for the disease being prevented. For example, the cumulative probability of developing colorectal cancer over full-life expectancy ranges between 4% and 6% (4), resulting in an extremely high percentage of people who will not benefit from prophylactic treatment unless the benefits extend to other health endpoints (3). The situation is different in certain genetically susceptible subgroups, such as in individuals with genetic mutations associated with hereditary nonpolyposis colon cancer (HNPCC) or familial adenomatous polypos (FAP) in whom lifetime risk for these diseases increases up to 70-90%, although these high-risk groups contribute only a small fraction of all cases of colorectal cancer. A second limitation to cancer chemoprevention in average- or low-risk populations concerns the logistic difficulty of studying cancer endpoints in large-scale prevention trials (3). Because of the relatively low annual risk of developing cancer in the general population, phase III chemoprevention trials must be much larger than either trials of aspirin in patients at high risk of cardiovascular disease or therapeutic trials in cancer patients already diagnosed with disease to have adequate statistical power (3). To date, it is unclear whether NSAIDs will be the primary agents for cancer chemoprevention in the future (5).

The current trend in cancer therapeutics is based on a molecular targeted design derived from preclinical *in vitro* and *in vivo* studies. Many *in vitro* studies have demonstrated

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the possible role of NSAIDs as a single agent to prevent cancer. Moreover, large epidemiological studies designed for other purposes have indicated that NSAIDs, such as aspirin, could have a beneficial influence on diminishing the development and growth of malignancies.

This review will discuss the anticancer molecular mechanisms associated with NSAIDs, their COX-independent mechanisms of action and their efficacy as chemopreventive and therapeutic agents in various epithelial malignancies.

Mechanisms of Action of NSAIDs

Despite the wide use of NSAIDs over the last century, their mechanism of action was not fully appreciated until 1971, when Vane demonstrated that the ability of NSAIDs to suppress inflammation rests primarily on their ability to inhibit the cyclooxygenase (COX) enzyme (also referred to as prostaglandin H (PGH) synthase) (6). Upon the discovery of COX, alternative enzyme forms were initially suggested. With the development of molecular biology, the discovery of two major cyclooxygenase genes (*COX-1* and *COX-2*) was heralded in 1990 (7).

COX-1 is constitutively expressed in platelets and in the normal gastrointestinal mucosa and is required for physiological processes such as gastrointestinal mucosa maintenance and platelet aggregation (8). 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 (9). Most recently, a splice variant of COX-1 mRNA, retaining intron 1, and given the names COX-3, COX-1b or COX-1v, has been described, which is most abundant in the cerebral cortex (10). This variant is selectively inhibited by acetaminophen in some animal models (11). However, in humans, an acetaminophen-sensitive COX-3 protein is not expressed because the retention of the intron results in the production of a truncated protein with a completely different amino acid sequence than COX-1 or COX-2 lacking acetaminophen sensitivity (11). COX-1 and COX-2 converts arachidonic acid to prostaglandin G₂ (PGG₂); the subsequent peroxidase reaction converts PGG₂ to PGH₂ (Figure 1), leading to the formation of biologically important prostanoids that coordinate signaling between the cell of origin (autocrine) and neighboring cells (paracrine) by binding to transmembrane G-protein-coupled receptors (3). The production of these potent signaling molecules is strictly regulated at the level of expression for COX-2 and at the level of catalysis for both COX-1 and COX-2 (12, 13). Their catalytic regulation depends on the availability of the substrate arachidonate and on peroxide, which is required for this catalysis. While both isoforms have comparable K_m values for arachidonate, COX-2 is activated at much lower peroxide concentrations than COX1 (14). Additional

regulatory mechanisms include the tissue specific expression of particular synthases, *i.e.* thromboxane A₂ (TXA₂) synthase in platelets, and preferential coupling of the COX isoforms to specific synthases. Non-selective NSAIDs inhibit both COX-1 (by irreversible acetylation) and COX-2 (by competitive inhibition) (15), but exhibit different capabilities of inhibiting COX isoforms 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 but inhibits COX-2 only at plasma concentrations higher than 0.5 mM (3, 16). Most 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 (such as celecoxib, rofecoxib, valdecoxib) by the World Health Organization, selectively inhibits COX-2, while sparing COX-1, thus avoiding the most serious gastrointestinal toxic effects associated with chronic high-dose NSAID use (17, 18). The marketing of the coxibs as "COX-2 inhibitors" has led to the assumption that there is a clear distinction between the COX-isotype selectivity of traditional NSAIDs and that of the coxibs. *In vitro* assays, however, demonstrated that there is a continuum of selectivity ranging from highly COX-1-selective (for example, ibuprofen and flurbiprofen) to highly COX-2-selective (for example, rofecoxib and lumiracoxibs) (Figure 2) (19, 20). The pharmacological 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 clinical context and drug dose. The determination of the lowest effective drug dose and the most critical period for administration is, in fact, one of the approaches used to improve the selectivity and reduce the toxicity of NSAIDs while achieving a specific pharmacological effect (3, 21). It was hypothesized that NSAIDs exert their anti-inflammatory and antitumor effects through inhibition of the inducible COX-2, while unwanted side-effects of these drugs such as damage to the gastric mucosa and gastrointestinal bleeding are thought to arise from the inhibition of the constitutive COX-1 (22). This hypothesis led to the development of coxibs. Indeed, highly selective COX-2 inhibitors retain the anti-inflammatory and antitumor effects of the NSAIDs while not interfering with COX-1 responsible for protection of the gastroduodenal mucosa from the effects of acid from the stomach (23). Therefore, coxibs were approved by the FDA, and as novel anti-inflammatory agents their use is associated with about a 50% reduction in gastrointestinal toxicity. Moreover, their potential for use as chemopreventives (3, 24) led to approval in December 1999 by the FDA for use in the prevention of colorectal polyp formation of patients with FAP. Unexpectedly, recent evaluation, as part of large-scale chemoprevention studies, showed that the improved risk

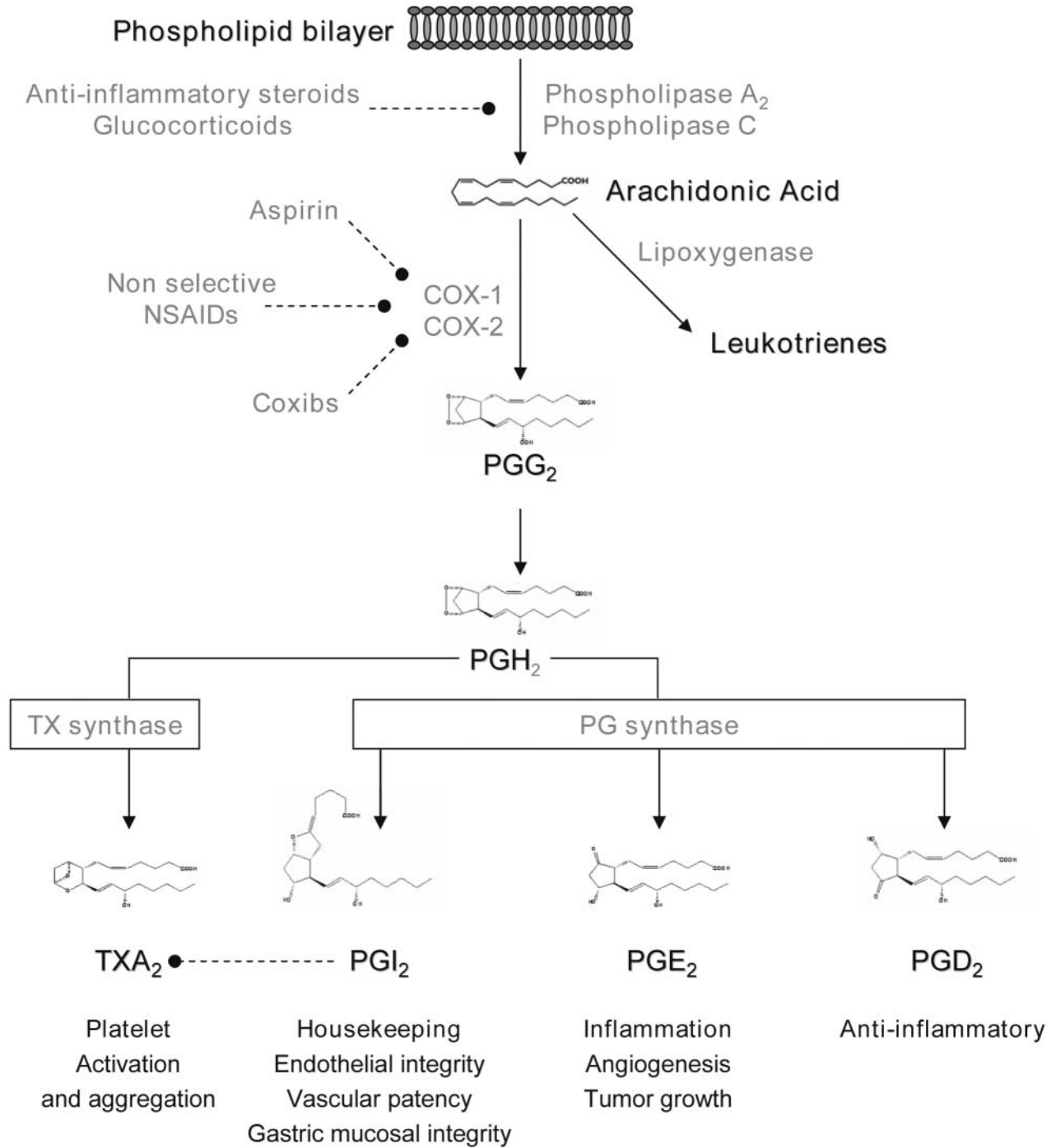


Figure 1. Overview of prostaglandin (PG) synthesis and main functions. Arachidonic acid is metabolized mainly through two major pathways: the lipoxygenase pathway, leading to formation of leukotrienes, and the cyclooxygenase (COX) pathway, leading to formation of prostaglandin (PGI₂, PGE₂, PGD₂) or thromboxane A₂ (TXA₂), each of which exerts its unique functions. NSAIDs, non-steroidal anti-inflammatory drugs; coxibs, COX-2 selective inhibitors.

profile for gastric toxicity of coxibs might occur at the expense of cardiovascular toxicity (25). On the advice of the Data Safety Monitoring Committee, the APPROVE (Adenomatous Polyp Prevention On Vioxx) trial, in fact,

was stopped early due to a significant excess of adverse cardiovascular events in the treatment groups. The safety profile of NSAIDs will be discussed later; however, we must keep in mind that early termination of the APPROVe trial

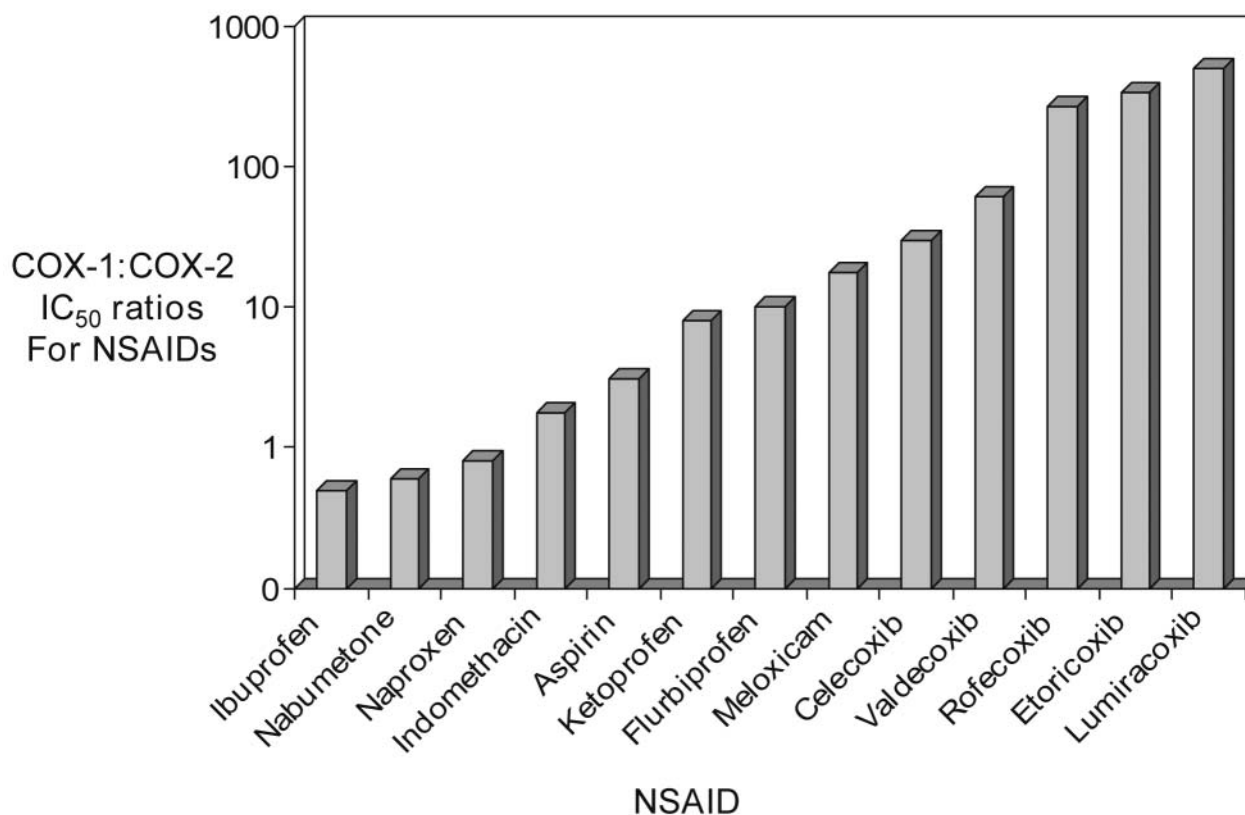


Figure 2. Range of cyclooxygenase-1 (COX-1):cyclooxygenase-2 (COX-2) selectivity for different non-steroidal anti-inflammatory drugs (NSAIDs) evaluated by COX-1 IC₅₀ to COX-2 IC₅₀ ratios. IC₅₀, concentration of drug necessary to inhibit the activity of COX-1 and COX-2 by 50%. A selectivity index ratio <1 indicates that the drug is more COX-1 selective, whereas a ratio >1 indicates the drug is more COX-2 selective (20).

was shortly followed by the announcement of the worldwide withdrawal of rofecoxib. The molecular mechanisms responsible for the effects of COX-2 inhibition on thrombus formation and the coagulation system cannot be reduced to a simple alteration of the balance of prostaglandin I₂ (PGI₂) and TXA₂ in endothelial cells and platelets, respectively, but is rather dependent on several other variables. A detailed analysis of the mechanisms involved in thrombotic complications in patients receiving coxibs fall outside the aims of this article and the reader is referred to recent reviews for further insights (26, 27). Nonetheless, all the above cited events boosted controversy concerning whether long-term use of coxibs may promote thrombosis or offset the cardiovascular benefits of low doses of aspirin (28). Accordingly, in August 2005, the Food and Drug Administration (FDA) Advisory Committee meeting modified the prescribing information for coxibs to include black-box warnings about cardiovascular as well as gastrointestinal risks. Future research on pharmacogenetics might help us better understand the risk-benefit balance in individuals and will help ensure that NSAIDs or coxibs are used in the most effective way for cancer prevention (29).

Experimental Evidence for Cancer Preventative Properties of NSAIDs

The link between inflammation and cancer was first suggested by Rudolph Virchow in 1863 when he demonstrated leucocytes in neoplastic tissue. Virchow's original hypothesis has been revisited by many research groups, and there is some evidence to corroborate inflammation-mediated oncogenesis (30, 31). The epidemiological data available are very impressive and show a clear association between chronic inflammatory conditions and subsequent malignant transformation in the inflamed tissue. In this context, COX isoforms, particularly COX-2, seem to play a pivotal role, being capable of inhibiting apoptosis, modulating cellular adhesion and motility, promoting angiogenesis and immunosuppression. Among the most potent inducers of COX-2 are the key proinflammatory cytokines IL-1 β and TNF- α .

Molecular mechanisms of COX-1 and COX-2 in carcinogenesis. Since NSAIDs are inhibitors of COX activity and PG formation, one would assume the prevention of

Table I. Downstream targets of PGE₂-mediated actions on cancer growth.

Target	Action	Ref.
EGFR	Stimulation of cell migration through increased PI3K-Akt signaling	Sheng <i>et al.</i> , 2001 (45) Buchanan <i>et al.</i> , 2003 (52) Pai <i>et al.</i> , 2002 (46) Torrance <i>et al.</i> , 2000 (53) Moran <i>et al.</i> , 2004 (54)
PPAR δ	Anti-apoptotic effects <i>via</i> PI3K-Akt signaling	Tsujii <i>et al.</i> , 1998 (49)
Bcl-2	Anti-apoptotic effects <i>via</i> increased NF- κ B transcriptional activity	Poligone and Baldwin, 2001 (50)
Ras	Induction of cell proliferation <i>via</i> signaling pathways such as the Raf/MEK/ERKs and PI3K/Akt	Kage <i>et al.</i> , 1999 (47) Gupta <i>et al.</i> , 2004 (48)
ERK2/JNK1	Increased expression of angiogenic factors (<i>i.e.</i> VEGF and bFGF)	Husain <i>et al.</i> , 2001 (55) Wang <i>et al.</i> , 2005 (56)

EGFR: epidermal growth factor receptor, PPAR δ : peroxisome proliferator activated receptors δ , VEGF: vascular endothelial growth factor, bFGF: basic fibroblast growth factor.

cancer by these drugs is dependent on the inhibition of PG formation. Indeed, the critical role of PGs in the development of cancer is supported by a considerable body of data. COX-2-derived PGE₂ is a proinflammatory bioactive lipid and is the major PG produced in many human solid tumors, including cancer of the colon (32), stomach (33) and breast (34). The only other COX-2 derived PG implicated in oncogenesis is TXA₂, which was reported to promote angiogenesis (35). Direct evidence supporting the notion that PGE₂ promotes tumor growth comes from the following observations. PGE₂ reversed NSAID-induced adenoma regression in *Apc*^{Min} mice (36). PGE₂ significantly enhanced colon carcinogen-induced tumor incidence and multiplicity in rats (37). Furthermore, PGE₂ accelerates intestinal adenoma growth in *Apc*^{Min} mice (38). In addition to studies with COX-1 and COX-2 knockout mice, some of the most compelling evidence is provided by experiments investigating PG receptors (EP) and the generation of EP-deficient mice (39). PGE₂ exerts its cellular effects by binding to its cognate receptors (EP1-4) that belong to a family of transmembrane G-protein coupled receptors. Mice with homozygous deletions in EP1 and EP4 receptors, but not EP3, were partially resistant to colon carcinogen-mediated induction of aberrant crypt foci (40, 41). EP2 disruption reduced the number and size of intestinal polyps in *APC* ^{Δ 716} knockout mice (42). Moreover, in carcinogen-treated wild-type mice, an EP1 receptor antagonist also reduced the incidence of aberrant crypt foci whereas *Apc*^{Min} mice treated with the same EP1 receptor antagonist and an EP4 receptor antagonist developed 57% and 69% fewer intestinal polyps, respectively, than untreated mice (40, 41). In addition to colorectal cancer, it has been shown that EP1,

2, and 4 receptors were elevated whereas EP3 receptor levels decreased in mammary tumors in COX-2-MMTV mice (43). Furthermore, an EP1 receptor antagonist was shown to reduce the tumor burden in a carcinogen-induced rat mammary model (44). Taken together, these findings may provide a rationale for the development of EP receptor antagonists which may offer an alternative to COX-2 selective inhibitors (39). Thus, PGE₂ promotes tumor growth by stimulating EP receptor signaling pathways and downstream targets that are involved in promoting cellular proliferation (45-48), inhibiting apoptosis (38, 49, 50), stimulating invasion and motility (45, 46, 51-54) and angiogenesis (55, 56) (Table I). Although sustained overexpression of COX-2 is an undiscussed critical event in carcinogenesis, the role of COX-1 and interactions between COX-1 and COX-2 in carcinogenesis have not been clarified. Modulation of the constitutive COX-1 gene may, indeed, play an important role, as suggested by studies of COX-1 knockout mice in the multiple intestinal neoplasia mouse model (57, 58). Notably, genetic deficiency of COX-1 produced a significant reduction in the development of intestinal tumors in this model. The absence of COX-1 may also reduce carcinogenesis in a mouse skin initiation/promotion model (57). Moreover, it should be noticed that the strongest effects of NSAIDs (nonselective in the majority of studies) in reducing cancer risk were usually reported in the gastrointestinal tract where COX-1 is constitutively produced (58).

COX-independent effects of NSAIDs. Several other mechanisms may be involved in the antineoplastic effects of NSAIDs, some of which may be independent of COX

expression and prostaglandin biosynthesis (59). For example, treatment of various tumor cell lines with celecoxib induces G1-phase arrest, which is accompanied by the reduced expression of cyclins A, B and D (60, 61). Molecular mechanisms involved in the cell cycle arrest induced by celecoxib treatment are only partially understood and seem to involve the inhibition of protein kinase B (PKB/Akt) or its upstream kinase phosphoinositide-dependent kinase 1 (PDK-1) (62, 63). Although many studies have reported that PKB is inhibited by celecoxib, whether celecoxib binds directly to PKB or acts by means of another celecoxib target, such as PDK-1, is presently unclear (59). However, 4-[5-(2,5-dimethylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene sulfonamide, which is a celecoxib analogue that lacks COX-2 inhibitory activity, also inhibits PDK-1 and PKB activity. Thus, these inhibitory effects may be independent of COX-2 (63).

NSAIDs may also exert their anticarcinogenic effect in various cancer cell lines through the induction of apoptosis (59). Apoptosis, or programmed cell death, can be induced by the extrinsic pathway through activation of death receptors or by the intrinsic pathway by means of the release of cytochrome c from the mitochondria (64). Evidence that the intrinsic apoptotic pathway appears to be activated by celecoxib (65) includes the observations that expression of the antiapoptotic proteins Bcl-2, Bcl-xL, Mcl-1, and survivin decreases after treatment of cancer cells with celecoxib, whereas expression of the proapoptotic protein Bad increases (59, 66), and rapid release of cytochrome c from mitochondria and the activation of Apaf-1 and caspases 3, 8, and 9 are observed (59, 65). In addition NSAIDs might influence apoptosis through different pathways, including: i) an increase in ceramide and subsequent release of proapoptotic proteins from the mitochondria *via* the formation of ceramide channels (59, 67); ii) inhibition of the activity of Ca^{2+} ATPase, so that the reuptake of Ca^{2+} from the cytosol is prevented, which elevates the free intracellular concentration of Ca^{2+} (68); iii) induction of 15-lipoxygenase 1 and increased production of proapoptotic molecules, such as 13-*S*-hydroxyoctadecadienoic acid (13-*S*-HODE) (69); or iv) changes in gene expression, as in the case of the NSAID activated gene-1 (*NAG-1*), a member of the TGF- β superfamily, which is involved in tumor progression and development (70, 71). Finally, overexpression of COX-2 in tumor cells may affect angiogenesis by the production of COX-2-derived eicosanoids (*i.e.* thromboxane A_2 , PGI_2 and PGE_2), which stimulate endothelial cell migration and angiogenesis by increasing the expression of VEGF and stimulating endothelial cell proliferation (59). Both mechanisms contribute to the formation of new blood vessels. Inhibition of COX-2 activity by coxibs reduces all these effects and leads to inhibition of angiogenesis and reduced tumor growth (61). COX-2-independent mechanisms that

contribute to the antiangiogenic effects of celecoxib have also been described, acting through inhibition of Egr-1 (72) or transcription factor Sp1 (73). In addition, NSAIDs can inhibit angiogenesis through increased endothelial cell apoptosis, inhibition of endothelial cell migration, recruitment of inflammatory cells and platelets, and/or TXA_2 -mediated effects, all of which have been associated with growth inhibition and attenuation of the metastatic potential of cancer cells (59).

Clinical studies and randomized trials of NSAIDs in cancer prevention and treatment. The potential antineoplastic properties of NSAIDs were discovered more than 20 years ago (1); since then, evidence has been accumulating, mainly derived from population-based observational studies, showing a reduced risk in cancer incidence for individuals consuming NSAIDs (74-81). In 1991 Thun *et al.* published an epidemiological prospective study on 662,424 adults, in which data on frequency and duration of aspirin use were collected and death rates from colon cancer were recorded through a period of 6 years. Participants consuming aspirin 16 or more times per month for at least one year had a reduced risk (RR) of developing colorectal cancer compared to those consuming fewer than 16 aspirin tablets a month (RR men: 0.60, RR women: 0.58), whereas no association was found between the use of acetaminophen and the risk of colon cancer (76). Approximately ten years later, Langman *et al.* reported the results from a case-control study using the United Kingdom general practice research database which enrolled 12,174 patients with a diagnosis of oesophageal, stomach, colonic, rectal, pancreatic, bladder, breast, lung or prostate cancer (78). A protective effect from NSAIDs against cancer of the oesophagus (RR 0.64, 95% CI 0.41 to 0.98), stomach (RR 0.51, 95% CI 0.33 to 0.79), colon (RR 0.76, 95% CI 0.58 to 1.00), and rectum (RR 0.75, 95% CI 0.49 to 1.14) was found with dose-related trends (78). In recent years, a growing body of evidence from pre-clinical and clinical studies has suggested a correlation between NSAID use and lower incidence of cancer. These results led NSAIDs being considered as potential antineoplastic agents for cancer prevention. Much of the clinical evidence in this field has been reached in colorectal cancer prevention (Table II), however other common tumors seem to be affected by NSAID treatment (Table III).

(i) *Familial adenomatous polyposis.* The evaluation of the effect of NSAIDs on colorectal cancer was initially examined in patients with familial adenomatous polyposis (FAP). In a study by Giardiello *et al.*, 22 patients with FAP received either 300 mg of sulindac per day for 9 months or placebo (82). A reduction in the number and the diameter of colorectal adenomas was observed in treated patients,

Table II. NSAID trials in colorectal cancer.

Study	Type of NSAID	Population setting	Study end-point (SE)	RR for the SE among NSAID consumers	P-value
Sporadic colorectal cancer					
Thun <i>et al.</i> , 1991 (76)	Aspirin	Patients with no diagnosis of cancer at study entry	Risk of dying from colorectal cancer	0.60 (men) 0.58 (women)	0.003
Giovanucci <i>et al.</i> , 1995 (89)	Aspirin	Patients with no diagnosis of cancer at study entry	Risk of diagnosing colorectal cancer	0.56 (women)	0.008
Langman <i>et al.</i> , 2000 (78)	Various	Patients with no diagnosis of cancer at study entry	Risk of diagnosing colorectal cancer	0.76 (colon) 0.75 (rectal)	0.05
Sandler <i>et al.</i> , 2003 (85)	Aspirin	Patients with radically resected colorectal cancer	Risk of diagnosing an adenoma	0.65	0.004
Baron <i>et al.</i> , 2003 (86)	Aspirin	Patients with resected colorectal adenomas	Risk of diagnosing an adenoma	0.81	0.04
Bertagnolli <i>et al.</i> , 2006 (93)	Celecoxib	Patients with resected colorectal adenomas	Risk of diagnosing an adenoma	0.55	<0.001
Arber <i>et al.</i> , 2006 (91)	Celecoxib	Patients with resected colorectal adenomas	Risk of diagnosing an adenoma	0.64	<0.001
FAP					
Giardiello <i>et al.</i> , 1993 (82)	Sulindac, high doses	22 patients with FAP	Reduction in polyp number	0.56	0.014
Giardiello <i>et al.</i> , 2002 (83)	Sulindac, low doses	41 patients with FAP	Reduction in polyp number	N/A	0.69
Steinbach <i>et al.</i> , 2000 (84)	Celecoxib	77 patients with FAP	Reduction in polyp number	0.72	0.003

Table III. NSAID trials in common types of cancer other than colorectal.

Study	Type of NSAID	Population setting	Study end-point (SE)	RR for the SE among NSAID consumers	P-value
Oesophageal cancer					
Langman <i>et al.</i> , 2000 (78)	Various	Patients with no diagnosis of cancer at study entry	Risk of diagnosing oesophageal cancer	0.64	0.05
Stomach cancer					
Langman <i>et al.</i> , 2000 (78)	Various	Patients with no diagnosis of cancer at study entry	Risk of diagnosing gastric cancer	0.51	0.05
Breast cancer					
Shen <i>et al.</i> , 2006 (96)	Various	Patients with radically resected breast cancer and COX-2 .8473 genotype	Risk of diagnosing breast cancer	0.70	0.02
Lung cancer					
Altorki <i>et al.</i> , 2003 (111)	Celecoxib	Neoadjuvant treatment with celecoxib/paclitaxel/carboplatin for NSCLC patients	Overall response rate	(65%)	N/A
Prostate cancer					
Mahmud <i>et al.</i> , 2006 (119)	Aspirin	Patients with no diagnosis of cancer at study entry	Risk of diagnosing prostate cancer	0.58	0.01
Smith <i>et al.</i> , 2006 (122)	Celecoxib	Prostate cancer patients with rising PSA previously treated with local therapy radical prostatectomy and/or radiation therapy	Post-treatment PSA Doubling time (PSADT) >200% of pre-treatment PSADT with no new metastases	0.75*	0.08

*Calculated from the percentage of patients experiencing a negative PSADT outcome in the placebo end celecoxib group.

however, since no complete regressions were seen, authors concluded that NSAID treatment cannot replace preventive surgery (82). Lower doses of sulindac were not able to show a significant prevention of adenoma development in FAP, as confirmed by another study of the same authors in which 41 patients were randomized to receive either 75 or 150 mg of sulindac twice a day or placebo for 4 years (83). On the other hand, Steinbach *et al.* analyzed the effects of a selective COX-2 inhibitor (celecoxib) in patients with FAP (84). In a double-blind study, 77 patients were randomly assigned to treatment with celecoxib (100 or 400 mg twice daily) or placebo for six months. Patients underwent colonoscopy at the beginning and at the end of the study and the number and size of polyps were determined from photographs and videotapes. After six months, patients receiving 400 mg of celecoxib twice a day had a 28% reduction in the mean number of colorectal polyps ($p=0.003$) and a 31% reduction in the polyp burden (*i.e.* the sum of polyp diameters) ($p=0.001$), as compared with reductions of 4.5% and 4.9%, respectively, in the placebo group (84). The reductions in the group receiving 100 mg of celecoxib twice a day compared to the placebo group were approximately 12% ($p=0.33$) and 15% ($p=0.09$), respectively. The incidence of adverse events was similar between the groups (84).

(ii) *Sporadic colorectal adenoma and cancer.* Adenomatous polyps are recognized as precancerous lesions for colorectal cancer development and NSAIDs have a potential role for reducing the incidence and recurrence of colorectal adenomas (83, 85, 86), acting through the effects reported above on COX-2, which is generally overexpressed in colorectal cancers and adenomas in humans (9, 87). Moreover initial observational studies and an extensive body of preclinical research demonstrated that colorectal cancer more than other cancer types can be affected by COX-2 inhibition, hence prompting clinicians to conduct prospective trials investigating the chemopreventive effects of NSAIDs against sporadic colorectal adenomas and cancer development (85-89). One such study showed that after a long period of aspirin consumption at doses corresponding to those advised for cardiovascular disease prevention the risk of diagnosing colorectal cancer in women was substantially reduced (89). This study was conducted within the Nurses' Health Study (551,651 person-years of follow-up), in which 331 new diagnoses of colorectal cancer were made. Women who consistently consumed aspirin (two or more tablets per week) were compared to "nonuser" women. After 9 years of follow-up, no appreciable reduction in colorectal cancer risk was seen, however a non-statistically significant risk reduction was observed after 10 to 19 years of aspirin consumption (RR 0.70; 95% CI, 0.41 to 1.20), which became statistically

significant after 20 years of treatment (RR 0.56; 95% CI, 0.36 to 0.90; $p=0.008$). The maximal benefit was seen when four to six tablets per week were consumed, whereas higher doses gave no additional advantage (89). In 2003, two randomized studies were published demonstrating the chemopreventive role of aspirin on sporadic colorectal adenoma occurrence (85, 86). In a randomized, double-blind trial by Sandler *et al.* 635 patients previously diagnosed with colorectal cancer were treated either with 325 mg of aspirin per day or placebo (85). Participants had to have a history of histologically documented colorectal cancer. Dukes' stage A or B1 cancer patients who had a curative resection were immediately recruitable. Patients with Dukes' stage B2 or C cancer were eligible if they had been free of disease for more than five years after radical resection. A basal colonoscopy with removal of all polyps was required within 4 months before enrolment in the study. At least one colonoscopy after a median time of 12.8 months was performed in 517 out of 635 randomized patients. Adenomas were found in 17% of patients in the aspirin arm compared to 27% in the placebo arm ($p=0.004$). The mean number of detected adenomas was also inferior in the aspirin arm (0.30 vs. 0.49, $p=0.003$). The aspirin/placebo RR for adenoma occurrence was 0.65 (95% CI, 0.46 to 0.91). Even the time interval between randomization and first detection of an adenoma was longer in the aspirin arm compared to the placebo arm ($p=0.022$). The study was closed early when the results were available at a planned interim analysis (85).

The other study was designed as a randomized, double-blind study in which 1121 patients with a history of histologically proven adenomatous polyps were treated with placebo, 81 mg of aspirin, or 325 mg of aspirin daily (86). Inclusion criteria were: colorectal adenomas removed within 16 months before enrolment and a complete colonoscopy performed within three months before enrolment with no colorectal polyps remaining. Follow-up colonoscopy was to be performed approximately three years after the baseline colonoscopy. The incidence of one or more adenomas was 47% in the placebo group, 38% in the group given 81 mg of aspirin per day, and 45% in the group given 325 mg of aspirin per day (global $p=0.04$), leading the authors to conclude that low-dose aspirin has a moderate chemopreventive effect on second adenoma occurrence (86).

All these studies were performed using non selective NSAIDs, mostly aspirin. Starting from 1999 new expectations were raised for the use of selective COX-2 inhibitors in colorectal cancer prevention. Three international, multicenter studies were launched in 1999 and 2000: the Adenoma Prevention with Celecoxib (APC), the APPROVe, and the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trials (25, 90-94). The aims were to assess the effects of coxibs on the formation of

adenomas and evaluate the possibility of overcoming the gastrointestinal adverse events of nonselective NSAIDs. As reported above, the APPROVe trial was stopped early due to a significant excess of adverse cardiovascular events in the treatment groups (25). In the PreSAP trial, 1561 participants who had had adenomas removed before enrolment were randomly assigned to receive celecoxib given daily in a single 400-mg dose or placebo. The primary outcome was detection of adenomas at either year 1 or 3 by colonoscopy. Overall, the three-year estimated cumulative rate of adenomas was 33.6% for those receiving celecoxib and 49.3% for those receiving placebo (RR, 0.64; 95% CI, 0.56 to 0.75; $p < 0.001$) (91, 92). Among those with any new adenoma detected, the mean size of the largest adenoma and the mean adenoma burden were significantly lower in the celecoxib than in the placebo group; the difference in the mean number of adenomas was not significant (91, 92).

In the APC trial, patients who had adenomas removed before study entry and had a high risk of recurrent adenomas on the basis of a history of either multiple adenomas or removal of a single adenoma more than 5 mm in diameter were randomly assigned to receive placebo or 200 mg or 400 mg of celecoxib twice daily. As in the PreSAP trial, follow-up colonoscopies were performed at 1 and 3 years after randomization. The estimated cumulative incidence of the detection of one or more adenomas by year 3 was 60.7% for patients receiving placebo, as compared with 43.2% for those receiving 200 mg of celecoxib twice a day (RR, 0.67; 95% CI, 0.59 to 0.77; $p < 0.001$) and 37.5% for those receiving 400 mg of celecoxib twice a day (RR, 0.55; 95% CI, 0.48 to 0.64; $p < 0.001$) (93).

An independent safety committee for both studies adjudicated and categorized serious cardiovascular events and then combined individual patient data from these long-term trials to improve the estimate of the cardiovascular risk associated with celecoxib compared with placebo (94). For APC and PreSAP combined, 83 patients experienced cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, or heart failure. The hazard ratio for this prespecified composite end-point was 2.6 (95% CI, 1.1 to 6.1) in patients taking 200 mg twice daily, 3.4 (95% CI, 1.5 to 7.9) in patients taking 400 mg twice daily in APC, and 1.3 (95% CI, 0.6 to 2.6) in patients taking 400 mg once daily in PreSAP (p for heterogeneity = 0.13 comparing the combined doses in APC with the dose in PreSAP). Overall, celecoxib showed a nearly 2-fold increased cardiovascular risk (94). In the APPROVe trial, patients were randomly assigned in a double-blind fashion to placebo or rofecoxib 25 mg/day (92). According to the main inclusion criteria (recently removed colorectal adenoma with histological confirmation), 2,587 patients were enrolled. As in the PreSAP and APC trial, a stratification by baseline use of aspirin for cardiovascular prevention was made. Colonoscopic second look had to take

place at year 1 and 3 from study entry. Results showed a reduced probability of detecting a new adenoma in the rofecoxib arm compared to placebo arm (41% vs. 55%, respectively, $p < 0.0001$, RR: 0.76). The p -value was also significant for risk reduction when a diagnosis of more advanced adenoma was considered ($p < 0.01$), with the preventive effect of rofecoxib being more evident in the first year of follow-up. However for rofecoxib an increased cardiovascular toxicity was documented as it was for the other selective COX-2 inhibitors.

These findings indicate that celecoxib is an effective agent for the prevention of colorectal adenomas but, due to the potential cardiovascular events, cannot be routinely recommended for this indication, although the trend for a dose-related increase in cardiovascular events raises the possibility that lower doses or other dose intervals may be associated with less cardiovascular risk (90, 94).

(iii) *Breast cancer*. Epidemiological investigations suggested that the regular use of NSAIDs such as aspirin or ibuprofen is related to a decrease in the risk of the breast cancer (57). However, the association between use of NSAIDs and breast cancer risk remains unclear. Recently, an *in vitro* study correlated the inhibition of COX-2 activity with reduced breast cancer cell proliferation, migration, invasion and matrix metalloproteinase (MMP) expression, suggesting that COX-2-dependent activity is a necessary component for cellular and molecular mechanisms of breast cancer cell motility and invasion (95). Further evidence for the role of COX-2 in breast cancer came from a recent population-based, case-control study, in which 1,067 breast cancer cases and 1,110 control individuals included in the Long Island Breast Cancer Study Project were genotyped to demonstrate that COX-2 polymorphisms may reduce overall breast cancer risk or risk for subtypes of breast cancer by modulating the inflammatory response and possibly interacting with aspirin or any NSAIDs use (96). The antitumor growth and antimetastatic actions of celecoxib were also investigated in a metastatic murine mammary cancer model demonstrating that this drug may be useful as an adjuvant therapy for breast cancer containing *p53* mutations due to its ability to both induce *p53*-independent mitochondria-mediated apoptosis and exert anti-angiogenic potentiality (97). Of interest, combined treatment with COX-1 and COX-2 specific inhibitors would exhibit synergistic effects against breast cancer *in vitro*, as combined treatment produced a significantly greater inhibition than single agents alone (98). Finally, we should take into account that PGs are capable of stimulating aromatase gene expression and thus estrogen biosynthesis. Given the importance of estrogen in the pathogenesis of breast cancer, the ability of aspirin and other NSAIDs to protect against breast cancer could vary according to hormone receptor

status (99). Indeed, encouraging results have been obtained using COX-2 inhibitors in combination with hormonal therapy. There are ongoing studies for the evaluation of selective COX-2 inhibitors in combination with cancer chemotherapy and hormonal therapies for the treatment of the different stages of breast cancer (100). In hormonal-sensitive breast cancer, exemestane plus celecoxib was compared to exemestane alone or letrozole alone in a neoadjuvant setting: complete responses were obtained only in the combination arm (101). The role of COX-2 inhibitors in combination with the standard treatment of advanced disease were also evaluated in a phase-2 study of 30 postmenopausal breast cancer patients with histologically proven metastatic disease, treated with exemestane and celecoxib (102). The results obtained show that combined therapy has promising activity and tolerability and support their use in phase III clinical trials of short duration treatments (102).

Many trials are currently evaluating the usefulness of combining several targeted therapies. Cooperation between COX-2 and epidermal growth factor receptor (EGFR) pathways in carcinogenesis is obvious as mentioned above. Combinational therapy with both inhibitors of these pathways to improve anti-cancer efficacy seems logical considering their molecular interactions. Indeed, celecoxib has been found to delay onset of tumors associated with epidermal growth factor receptor-2 (HER-2) in animal models (103). However, despite the promising results of these two studies, further evidence is needed before COX-2 inhibitors can be routinely used for cancer treatment.

(iv) *Lung cancer.* Synergistic anticancer effects have been demonstrated in preclinical models of lung cancer using a combination of NSAIDs and old generation chemotherapeutic drugs (such as cisplatin, cyclophosphamide, and etoposide) (104-106). A synergistic effect was also demonstrated for the combination of sulindac with relatively new chemotherapeutic agents, such as paclitaxel and docetaxel, using an *in vitro* model of human non-small cell lung cancer (NSCLC) cell lines (107).

Recently, clinical studies have demonstrated a reduction of the risk for lung cancer with NSAID treatment (108, 109). In a case-control study by Harris *et al.* (108), comparing lung cancer patients and heavy smoker controls, a daily intake of NSAIDs for at least 2 years prior to interview was associated with a 68% reduction in the relative risk of lung cancer (108). In another case-control study by Muscat *et al.* (109), the relative risk estimate of lung carcinoma associated with using NSAIDs three times a week or more for one or more years demonstrated an RR of 0.68 for lung cancer development (109). The preventive effect was significant only amongst former or current smokers, and there was no difference between various types

of NSAIDs. Prompted by these positive findings, clinical trials were designed to determine the efficacy of a combination of chemotherapeutic agents with selective COX-2 inhibitors. Results are still preliminary but promising. For example, The THO-0054 phase II trial combined celecoxib with docetaxel in patients with recurrent, previously treated NSCLC. A decline in the levels of the major urinary metabolite of PGE₂ was observed indicating that celecoxib inhibits COX-2, but the overall response rate and median survival were similar to that observed with docetaxel alone (110). These results led the authors to conclude that combining celecoxib with docetaxel, at least at the doses and schedule employed in their study, does not improve survival in unselected patients with recurrent, previously treated NSCLC, although there might be a subset of patients with a marked decline in urinary PGE₂ metabolite who might take advantage of such therapeutic strategy (110). Different results were observed in another study by Altorki *et al.* in which 29 patients with stages IB to IIIA NSCLC were preoperatively treated with two cycles of paclitaxel and carboplatin, associated with daily celecoxib (paclitaxel 225 mg/m² and carboplatin AUC 6 on day 1, cycles repeated every 21 days, celecoxib 400 mg twice daily) (111). In this study, in fact, the observed overall clinical response rate was remarkably high (65% overall response rate: 48% partial responses and 17% complete responses) and superior to the historically reported response rates with standard treatment (111).

(v) *Prostate cancer.* Similar to prostate cancer in humans, prostate malignancies in a transgenic adenocarcinoma of the mouse prostate model (TRAMP) progress from precursor intraepithelial lesions to invasive carcinoma and to metastatic disease. Treatment of mice that develop prostate cancer with celecoxib results in suppression of tumor growth and decreased metastatic spread of disease (112, 113).

To date, clinical evidence for an association between NSAID use and RR for prostate cancer are still conflicting. Indeed, two recently published studies denied any decrease of prostate cancer risk in individuals under regular aspirin use (114, 115), but Leitzmann and colleagues suggested that although regular aspirin use is not likely to prevent the incidence of total prostate cancer, a possible benefit of frequent aspirin use on risk of developing metastatic prostate cancer cannot be excluded (116).

Other studies involving NSAIDs and prostate cancer suggested that the former may have a preventive effect. A multicentric cohort of over 90,000 men, in fact, demonstrated a 24% reduction in the risk for prostate cancer in patients under regular aspirin daily intake in the year preceding the study (117). Other relatively large case-control studies demonstrated an even more significant reduction in the risk of prostate cancer (118-121).

The availability of selective COX-2 inhibitors prompted the design of a phase III randomized study in which patients with rising serum PSA, after radical prostatectomy or radiotherapy, were assigned to receive celecoxib (800 mg/day) or placebo (122). Treatment continued until disease progression or until serious adverse event. Unfortunately, the study was terminated when the cardiovascular safety of celecoxib prompted review of ongoing clinical studies. Although the primary efficacy objective was not met, the study showed that compared with placebo, celecoxib significantly reduced mean PSA. Questions still remain as to whether or not these effects will correlate with an actual decrease in clinical recurrence of prostate cancer (122, 123).

Safety profile of NSAIDs and COX-2 inhibitors. Non selective NSAIDs that block both COX isoforms are associated with a spectrum of toxic effects due to their irritation of the gastric mucosa (124), inhibition of platelet function (16), and impairment of hepatic and renal function (125). Inhibition of COX-1 is associated with a slight elevation in the risk of toxicity to those organs where its constitutive expression maintains homeostasis (*i.e.* stomach, kidney) and may also reduce the production of TXA₂ in platelets. In contrast to COX-1, the COX-2 isoform catalyzes production of prostaglandins in response to pro-inflammatory signals. Selective COX-2 blockade inhibits PGE₂ and PGI₂ but has no effect on platelet TXA₂ (16). While serious adverse events occur infrequently with usual analgesic doses, higher dosages and/or the long-term use of aspirin and other NSAIDs may cause gastrointestinal ulceration and bleeding in a small percentage of patients (15).

Coxibs were developed for the express purpose of further reducing the already small risk of COX-1-related side-effects while maintaining a high degree of anti-inflammatory activity. However, predicting the consequences of COX-2 inhibition on cardiovascular disease is not a straightforward proposition. COX-2 inhibition has several effects that could increase the risk of cardiovascular disease, including reducing prostacyclin levels, increasing blood pressure, decreasing angiogenesis (126-133), and destabilizing plaque (134).

Rofecoxib is a selective COX-2 inhibitor that has been shown to be associated with significantly fewer gastrointestinal adverse events than NSAIDs (23). In one trial (23), there were more cardiovascular events among patients given a high dose of rofecoxib than among those given naproxen, an NSAID with platelet-inhibiting properties of unclear clinical relevance (127, 128, 135-137). Pooled data from other randomized trials have not shown a significant difference in cardiovascular risk between rofecoxib and placebo or other nonselective NSAIDs (127, 128, 137).

Observational studies have provided conflicting data on the association of rofecoxib with cardiovascular risk: some

studies suggested that there was no effect, some suggested that the risk was increased only at high doses, and others indicated a possible increase in the risk of cardiovascular events at standard or unspecified doses (129, 137-142). The APPROVe and APC Trials were designed to evaluate the hypothesis that treatment with coxibs would reduce the risk of recurrent adenomatous polyps among patients with a history of colorectal adenomas. Potential thrombotic events were adjudicated by an independent committee and all safety data were monitored by an external safety-monitoring committee; a dose-related increase in cardiovascular risk was observed with either celecoxib or rofecoxib. On the basis of these observations, the data and safety monitoring board recommended early termination of the APPROVe trial, shortly followed by the announcement of the worldwide withdrawal of rofecoxib.

Conclusion

The role of NSAIDs in cancer prevention is now established, but the clinical use of these drugs as chemopreventive agents is limited, since there are still many open questions about the optimal drug, dose, duration of therapy and knowledge about the mechanism(s) by which these drugs act. A chemopreventive drug should have an efficacy about 100% and an ideal safety profile, in this way these agents might be prescribed to healthy individuals. In this respect, the recent data on cardiovascular toxicity of coxibs limit the use of these drugs in chemoprevention, and sulindac or celecoxib are approved to suppress the growth of colorectal adenomatous polyps only in FAP patients.

Clearly, the risk *versus* the benefit of any therapeutic intervention needs to be cautiously considered. In those individuals for whom we can predict an almost certain recurrence of disease, as in the case of FAP, the benefit of a chemopreventive drug might justify its use even in the presence of adverse effects. On the other hand, the likelihood of serious gastrointestinal or cardiovascular toxicity of NSAIDs is probably too high to recommend their use in low-risk patients. As our ability to predict the course of a given disease in a given individual improves, the aggressiveness of our therapeutic intervention can be tailored so as to maximize the beneficial effects while reducing the adverse effects of any intervention we recommend.

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