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## Aspirin Use and Potential Mechanisms for Colorectal Cancer Prevention

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### Introduction

Disease prevention is receiving more attention in the United States today. This welcome trend is largely a result of two economic forces: The increased level of support for prevention research by the National Institutes of Health (Healthy People 2000), and the potential payoff of prevention research in cutting the health care costs of managing patients with end-stage diseases. Indeed, the efficacy of preventive measures for many chronic diseases (such as heart disease and cancer) are being evaluated with the full expectation that mortality and morbidity—and perhaps health care costs—will decrease.

One example of recent cancer prevention research indicates that there may be a 40–50% reduction in mortality from colorectal cancer in persons who take aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs)<sup>1</sup> on a regular basis. It is clear that an effect of this magnitude would have a significant impact on both the number of lives saved and on the health care dollars recovered. Colorectal cancer is a major cause of death in Western civilizations, claiming about 55,000 lives in the United States this year alone. Americans have a 1 in 20 lifetime risk of developing this disease, and ~ 1 in 10 have a family member who develops colorectal cancer.

Initially, it was hoped that early detection would reduce these high numbers. However, despite recent evidence that early detection by fecal occult blood tests and flexible sigmoidoscopy can decrease the risk of colorectal cancer death by 20–30%, most people do not undergo appropriate screening. Therefore, a number of laboratories have initiated research efforts focused on understanding the molecular basis of the potential chemoprotective effects resulting from aspirin and other NSAID use. It is hoped that this approach, in conjunction with more rigorous clinical trials, will lead to a rationale design for future colorectal cancer prevention regimens. This Perspectives article considers both the clinical and the basic re-

search efforts underway this year, the 100th anniversary of the discovery of aspirin.

### Clinical studies on colorectal cancer risk reduction in humans

Several human studies have evaluated the effect of aspirin and NSAID use on the relative risk of colorectal cancer (most of these evaluated the effect of aspirin use, therefore there is much less data on nonaspirin NSAIDs (1). The majority of these studies are observational in nature, generating little information regarding the most effective dose and duration of drug use. Currently, randomized controlled trials are underway to determine the effectiveness of aspirin use on recurrence of colorectal adenomas. These trials are designed to determine the most effective dose of aspirin and the appropriate duration of therapy.

There are concerns about the safety of long term aspirin use in humans. Long term aspirin use results in an increased risk of gastrointestinal bleeding, even at relatively low doses of drug. These side effects tend to increase with the age of the population. Depending on the dose and duration of drug required in the target population, the side effects of a chemoprotective agent must be low to insure compliance and to achieve the desired result, since the absolute risk of colorectal cancer in the general population is quite low. Because of the gastrointestinal and neurological related side effects attributed to sustained aspirin use, there is an enormous burden of proof required to document the efficacy of these drugs as chemoprotective agents for use in the general population. On the other hand, if high-risk populations can be readily identified then the use of these agents in high-risk patients may be more reasonable, because of a much more favorable risk-to-benefit ratio.

There have been several observational studies of the effects of exposure to NSAIDs (usually aspirin) and the subsequent development of colorectal cancer (1). All but one of these demonstrated a protective effect of NSAIDs against colorectal cancer. These studies were performed in a variety of settings in the United States and in Australia, using both colorectal cancer occurrence and mortality as the outcomes of interest. In most of the studies, exposure to NSAIDs was measured by interview, although in one study, computerized pharmacy records were used to measure NSAID exposure (2). In the Nurses Health Study (3) a protective effect was seen only after several years of aspirin use. A hospital-based study also demonstrated increasing protection with longer periods of use. Similar studies have revealed a protective effect of NSAIDs in relation to adenomatous polyp detection (4). Additionally, a small number of observational studies have shown a significant risk reduction with use of nonaspirin NSAIDs (1).

The most definitive evidence of benefit can only come from a randomized controlled trial. The effect of aspirin use on the development of colorectal cancer has been assessed in the set-

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1. Abbreviations used in this paper: COX-2, cyclooxygenase-2; FAP, familial adenomatous polyposis; NSAIDs, nonsteroidal antiinflammatory drugs.

ting of a clinical trial on aspirin for prevention of myocardial infarction. A secondary analysis of this study of male physicians randomized to placebo or aspirin 325 mg every other day demonstrated no protective effect against the development of colorectal cancer (5). Although colorectal cancer was not the primary endpoint of the original study, this was not likely to affect the results. However, it is possible that certain characteristics of the study group (such as diet, exercise regimen, age, and gender) or the relatively low dose of aspirin could obscure a protective effect.

Unfortunately, the frequency of colorectal cancer is too low to make it feasible to conduct a large scale randomized clinical trial (because of the cost and time required to complete such a study). Therefore, more definitive recommendations concerning aspirin use will likely be based on the results of ongoing randomized clinical trials of aspirin use that employ adenomatous polyp incidence as an intermediate endpoint. This multicenter study is testing the effect of aspirin at one of two doses versus placebo on the development of adenomatous polyps among patients who have undergone previous colonoscopy with polypectomy. Results of these studies should be available within the next 2 yr.

#### *NSAID use and reduction of adenoma size and number in FAP patients*

Familial adenomatous polyposis (FAP) is a disease inherited in an autosomal dominant fashion with variable phenotypic expression. It is associated with an increased risk of colorectal cancer at a young age. FAP is responsible for only ~ 1% of colorectal carcinomas detected in the general population. The genetic mutation responsible for this disease is in the adenomatous polyposis coli (APC) gene. Somatic mutations in the APC gene have been reported in up to 50% of spontaneous colorectal cancers examined (6, 7). Waddell and Loughry (8) first reported that sulindac use led to regression of rectal adenomas in four patients with FAP. After Dr. Waddell's initial account, several cases were reported describing adenoma resolution in FAP patients taking sulindac (4). The first randomized, placebo-controlled, double-blinded, crossover study of sulindac use in FAP patients was reported by Labayle et al. (9). This study was carried out in nine patients who received sulindac at a dose of 300 mg per day, or placebo during two 4 mo periods separated by a 1-mo washout phase. With sulindac treatment, a complete regression of polyps was noted in all patients. Giardiello et al. (10) conducted another randomized, double-blinded, placebo-controlled trial in 40 patients with FAP. Patients received oral sulindac (300 mg per day) or placebo for 9 mo. In the sulindac group, following 9 mo of treatment, the polyp number decreased by 44% of baseline levels ( $P = 0.014$ ) and polyp size by 35% ( $P = 0.001$ ). No patient was found to have complete resolution of adenomas. Lastly, Nugent et al. (10a) reported a randomized controlled trial of sulindac in 24 FAP patients. In this study, sulindac treatment caused a statistically significant reduction in rectal polyp count; however, the reduction in duodenal polyp count was not significant. These studies, collectively, indicate that the NSAID sulindac has a significant effect on polyp regression in FAP patients.

#### *Potential mechanisms for chemoprevention of intestinal tumors by aspirin and other NSAIDs*

What is the mechanism(s) of action for these anticarcinogenic effects? It is likely that these mechanisms are related, at least

in part, to those underlying the antiinflammatory properties of NSAIDs, that is, their ability to inhibit the cyclooxygenase enzymes. These enzymes catalyze key steps in the conversion of arachidonic acid to endoperoxide (PG<sub>2</sub>H), which is a substrate for a variety of prostaglandin synthases that, in turn, catalyze the formation of prostaglandins and other eicosanoids (Fig. 1). Two isoforms of cyclooxygenase have been identified to date, each possessing similar activities, but differing in expression characteristics and inhibition profiles by NSAIDs. Cyclooxygenase-1 (COX-1) was purified to homogeneity from bovine vesicular glands in 1976 (11). COX-1 mRNA and protein are expressed constitutively in many tissues. Early reports indicated the likely presence of an inducible cyclooxygenase activity whose induction could be blocked by treatment with glucocorticoids (12). A second, inducible isoform of cyclooxygenase, referred to as cyclooxygenase-2 (COX-2) was independently cloned by two groups (13, 14). COX-2 expression is affected by a number of extracellular and intracellular stimuli (15). The formation of COX-2 protein parallels the increase in prostaglandin production after stimulation with mitogens or tumor promoters in a wide variety of cell types. Prostacyclin production increases rapidly in rat intestinal epithelial (RIE) cells after stimulation with mitogens such as TGF $\alpha$  or EGF (16). The regulation of COX-2 expression is a key step in modulating prostacyclin production in these cells. After mitogenic stimulation, the level of COX-2 mRNA increases rapidly within 30 min in a cycloheximide independent fashion (17) remaining elevated for 6–8 h before rapidly declining to baseline levels within 24 h (18). In fact, COX-2 mRNA levels are super-induced in the presence of cycloheximide, providing a classic example of immediate early or early growth response gene activation.

Does dysregulation of COX-2 expression coincide with development of gastrointestinal malignancy? We have previously reported increased COX-2 expression in human colorectal adenocarcinomas when compared to normal adjacent colonic mucosa (19); these findings have been confirmed by other investigators using different techniques and patient populations (20, 21). Additionally, we have also observed markedly ele-

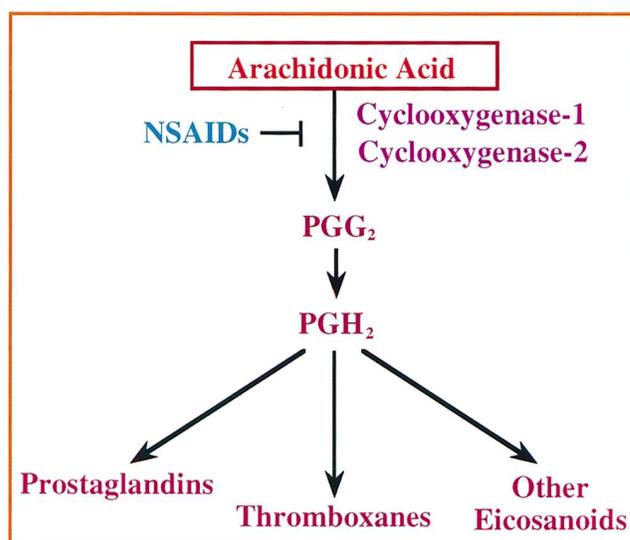


Figure 1. Arachidonic acid metabolism.

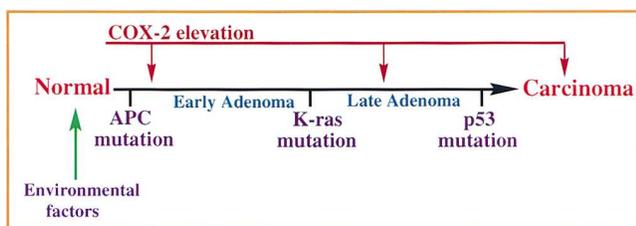


Figure 2. Working hypothesis for the potential role of COX-2 in colorectal carcinogenesis.

vated levels of COX-2 mRNA and protein in intestinal tumors that develop in rodents after carcinogen treatment (22) and in adenomas taken from *Min* mice (23). When intestinal epithelial cells are forced to express COX-2 constitutively they develop an altered phenotype that includes changes in their adhesion properties and a resistance to undergo programmed cell death (24). Both of these phenotypic changes are consistent with an increased tumorigenic potential.

We have developed a working hypothesis for the involvement of COX-2 in colorectal carcinogenesis, which is shown in Fig. 2. COX-2 expression has been detected in 80–90% of colorectal adenocarcinomas and in 40–50% of premalignant adenomas. From this data it appears that elevation of COX-2 expression is secondary to other initiating events, such as mutations of the APC gene or possibly mutations of other genes. Cells expressing high levels of COX-2 develop alterations in their adhesion to extracellular matrix and are resistant to undergo apoptosis, both of which could lead to continued adenoma growth.

Our observation of elevated COX-2 expression in three different models of colorectal carcinogenesis have led us to consider the possibility that COX-2 expression may be related to colorectal tumorigenesis in a causal way. Work by two independent groups has shown a reduction in tumor multiplicity in *Min* mice treated with sulindac or piroxicam, both potent cyclooxygenase inhibitors (25, 26). A recent study demonstrated a 40% reduction in aberrant crypt formation in carcinogen-treated rats who were given a selective COX-2 inhibitor (27). Another study has provided genetic evidence that directly links COX-2 expression to intestinal tumor promotion (28). This report shows that APC<sup>Δ716/+</sup> mice, which develop hundreds of tumors per intestine, bred with COX-2 null mice have a 80–90% reduction in tumor multiplicity in the homozygous COX-2 null offspring. These results suggest that: (a) COX-2 may act as a tumor promoter in the intestine, and (b) increased levels of COX-2 expression may result directly or indirectly from disruption of the APC gene.

Recently, a new class of NSAIDs has been developed that is highly selective for inhibition of the COX-2 enzyme, but lacks inhibition of COX-1 (29, 30). These drugs were primarily developed as antiinflammatory agents that would lessen the gastrointestinal side effects caused by inhibition of COX-1. Therefore, COX-2 selective inhibitors have potential for use as chemopreventive agents because they are effective in inhibiting tumor growth in animal studies (27, 28, 31).

#### Cyclooxygenase-independent mechanisms

Epidemiologic data strongly support the chemoprotective effects of NSAIDs on gastrointestinal malignancies, while the

data supporting their benefit in other solid tumors are weak or nonexistent. The precise mechanism by which NSAIDs prevent and/or cause regression of colorectal tumors is not known. Despite different chemical structures, inhibition profiles and drug half-lives, all NSAIDs in clinical use possess cyclooxygenase inhibitory activity. Some investigators have reported effects of NSAIDs that are not likely because of their inhibition of cyclooxygenase activity. For example, certain NSAIDs induce apoptosis and alter expression of cell cycle regulatory genes (32–34) in some cell lines when administered at relatively high concentrations (200–1,000 μM). Some of these experimental results have clearly ruled out the involvement of cyclooxygenase enzymes in the drug effects observed. However, almost any drug given to humans can have multiple effects depending on the dosage and duration of therapy. Since the introduction of widespread NSAID therapy in humans, several reports have indicated a wide range of effects on biological systems. However, it seems clear that their antiinflammatory and analgesic properties are most likely related to their ability to inhibit cyclooxygenase enzymes. The deleterious effects of nonselective NSAIDs on the gastrointestinal tract are widely known and include gastric erosions, ulcerations and blood loss (35), and are postulated to result from inhibition of COX-1 (36) (although some of these could arise from other mechanisms). Most investigators assume that the clinical effects and/or side effects of NSAIDs are probably related to their ability to inhibit cyclooxygenase enzymes. Certainly, this class of drugs affects biochemical pathways unrelated to cyclooxygenase enzymes and these effects are likely to occur in a dose-dependent fashion (some effects occurring only at toxic doses).

There is a groundswell of interest developing for the wholesale use of NSAID-like drugs for prevention of many types of cancer, including breast cancer (37). This must be approached carefully, because some epidemiologic data suggest that aspirin use in humans may not provide protective effects against breast cancer (38). Collectively, the current epidemiologic data indicate that the most significant benefits are obtained in prevention of malignancies of the gastrointestinal tract, including colon, gastric, and esophageal cancer (39).

#### Summary and implications for cancer prevention

Cardiovascular events are clearly reduced in patients using low dose aspirin after myocardial infarction. However, despite the fact that aspirin is widely recommended as secondary prophylaxis for the prevention of recurrent myocardial infarction and cerebrovascular accidents (40), the role of aspirin in primary prophylaxis of serious vascular disease is less clear (41). Two randomized controlled trials of aspirin prophylaxis for myocardial infarction have been performed among male physicians in the United States and United Kingdom (42, 43). The American trial of 325 mg of aspirin every other day and placebo demonstrated a 44% decrease in fatal and nonfatal myocardial infarctions. However, total cardiovascular deaths were not affected primarily because there was an increase in sudden death among those on aspirin (42). The British study demonstrated no protection against myocardial infarction using 500 mg of aspirin daily (43). There are no data from randomized trials of aspirin prophylaxis among women, although a large prospective cohort study demonstrated a protective effect of aspirin taken 1–6 times a week (44).

Preventive measures must meet a very high standard for

safety as well as efficacy. Data from observational studies and randomized controlled trials has indicated that there are risks from aspirin use that are evident even at small doses. In the randomized trials of primary prevention of myocardial infarction, there was an increase of strokes in both the United States and United Kingdom studies; however, the effect did not reach statistical significance in either study. Gastrointestinal conditions such as dyspepsia, peptic ulcer disease, and GI hemorrhage are exacerbated by aspirin and other nonsteroidal anti-inflammatory drugs (45). There appears to be a dose-related increased risk of peptic ulcer disease and gastrointestinal bleeding on prophylactic doses of aspirin. In the United States Physicians' Health Study, among those using 325 mg of aspirin every other day, there was a small excess (3 per 10,000 person years) of upper gastrointestinal bleeding. However, in studies using larger or more frequent doses of aspirin, there is an increased risk for peptic ulcer disease and significant gastrointestinal bleeding (46). The excess risk for gastrointestinal bleeding among those on aspirin (75 mg per day) for primary prophylaxis of stroke was 26/10,000 person years. The concerns of the efficacy and safety of aspirin led the United States Preventive Services Task Force to recommend neither for nor against the routine use of aspirin as primary prophylaxis against myocardial infarction. Because the risk of myocardial infarction is much greater among those individuals with a previous myocardial infarction there is little controversy about the role of aspirin for secondary prophylaxis. Thus, before a chemopreventative agent can be recommended as routine prophylaxis for colorectal cancer, it must have a safety profile that meets or exceeds that of low dose aspirin. Importantly, the risk-benefit ratio for chemoprevention of colorectal cancer would likely improve if groups could be easily identified that are at high risk for the subsequent development of colorectal cancer. As discovery and testing for colorectal cancer genes become more widely available, the identification of cohorts at high risk for developing cancer is more likely. Additionally, if agents were available that lack platelet effects with far less gastrointestinal toxicity (such as, potentially, the selective COX-2 inhibitors), the risk to benefit ratio might be favorably affected. However, the safety profile for these new drugs has not been established, a necessary step before they can be considered for widespread cancer prevention use.

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