Non-Steroidal Anti-Inflammatory Drugs and Selective Apoptotic Anti-Neoplastic Drugs in the Prevention of Colorectal Cancer: The Role of Super Aspirins

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There is increasing evidence to suggest that aspirin and other non-steroidal anti-inflammatory drugs reduce the risk of colorectal cancer. This observation is supported by animal studies that show fewer tumors per animal and fewer animals with tumors after administration of several different NSAIDs. Intervention data in familial adenomatous polyposis have established that the effect is exerted on the process of human colonic adenoma formation. Supportive evidence in sporadic colorectal neoplasia, derived from 22 of 24 studies (both case-control and cohort), found a reduced risk in men and women for cancers of the colon and the rectum and for both aspirin and the other NSAIDs. Earlier detection of lesions as a result of drug-induced bleeding does not seem to account for these findings. Although the molecular mechanism responsible for the chemopreventive action of this class of drugs is not yet completely understood, the protection may affect several pathways including both cell cycle arrest and induction of apoptosis.

In the third millennium the question is not if but how. Based on the consistency of epidemiological, clinical and experimental data, the association between regular long-term aspirin or NSAIDs intake and a decreased death rate from colorectal cancer is sound and there is no need for further placebo trials. At the same time, despite this consistency there is no clear data on the dose, duration or frequency of use for cancer-preventive activity.

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Cancer chemoprevention refers to the inhibition or reversal of carcinogenesis by intervention with natural or pharmacological agents. The discovery of the potential chemopreventive activity of non-steroidal anti-inflammatory drugs in sporadic human colorectal neoplasia is clearly a milestone, but the subject of chemoprevention is still under extensive ongoing investigation. We will review the current data on the biochemical and clinical influence of NSAIDs and selective apoptotic anti-neoplastic drugs on sporadic and familial colorectal neoplasia.

Worldwide, colorectal cancer is a major health problem, with 783,000 new cases per year representing about 9% of all cancers. It is the second leading cause of cancer death in the United States, with over 129,000 newly diagnosed patients and 57,000 deaths estimated in 1999 [1]. In Israel it is a leading cause of cancer death [2].

The evolving concept of the adenoma-carcinoma sequence, known for over 20 years, was investigated by the Hopkins' group, which described the molecular basis of this progression [3]. This paradigm is now well established, and the strongest clinical evidence is that patients who are maintained adenoma free by polypectomy are generally kept cancer free [4]. With a long latency period (a decade or more) there is now increasing scientific and clinical interest in prevention of colorectal cancer. Two important preventive measures are under active investigation: one is surveillance for early detection and removal of colorectal adenomas, and the other is interference with neoplastic formation and progression by chemopreventive agents. Among the few compounds that have been shown to be useful *in vivo* as chemopreventive agents are NSAIDs [5].

The relationship between NSAID and colorectal cancer is intriguing and comprehensive and, like many seminal findings in medicine, was discovered accidentally. Alert physicians noted that patients with familial adenomatous polyposis coli receiving NSAIDs as an anti-inflammatory treatment also experienced a regression of a variety of polyps. Several epidemiological studies have shown that aspirin and other NSAIDs may reduce the incidence of colorectal cancer [for reviews see 6–10]. Moreover, there are several lines of evidence suggesting that NSAIDs reduce the incidence and mortality from colorectal neoplasia. These include: a) more than 50 animal studies where NSAIDs were shown to prevent carcinogen-induced colorectal cancer [for

NSAIDs = non-steroidal anti-inflammatory drugs

reviews see 5,11,12]; b) FAP patients in whom sulindac treatment induced a dramatic regression of adenomas [13–15]; and c) the sporadic population in which regular long-term use of aspirin or NSAIDs was shown by 22 of 24 epidemiological studies to be associated with a 40–50% decreased death rate from colorectal cancer [for reviews see 6–10,16].

Animal model

More than 50 animal studies have demonstrated the protective effect of NSAIDs in rodents [for reviews see 5,11,12]. Animals treated with different NSAIDs and a colon carcinogen developed fewer colonic tumors than the control group treated with the carcinogen alone. These experiments clearly indicated that different members of the NSAID class consistently prevented carcinogen-induced colonic carcinogenesis in rodents up to 14 weeks after carcinogen administration.

In recent years studies using transgenic mice have recapitulated these findings. Of special importance were studies using the Multiple intestinal neoplasia mouse model. This strain was initially induced by germ line mutagenesis using the alkylating agent ethylnitrosourea [12]. These mice demonstrated mutations in the *APC* tumor suppressor gene, and because of their phenotypic similarity to FAP in humans this model was used in many subsequent chemopreventive studies. Different NSAIDs exerted several important effects both in Min mice and in other animal models, inhibiting adenoma formation and reducing the tumor burden [12].

Human studies

Twenty years ago, Waddell and Loughry from Denver, Colorado [13] were the first physicians to report the regression of various tumors in FAP patients receiving NSAIDs as anti-inflammatory treatment. This subsequently led to about 25 small prospective intervention studies using sulindac (randomized and non-randomized) in more than 150 patients [for reviews see 13–15]. Common to all these trials were the following observations: a dramatic reduction in the number and size of polyps; decreased formation of new adenomas; the beneficial effect is not influenced by the initial polyp burden (size and number); and long-term treatment is needed since adenomas frequently recur after discontinuation of the drug.

In seven controlled trials in 83 FAP patients the results were generally consistent with the previous studies [for reviews see 14,15]. In the classical study of Giardiello et al. [14], a double-blind randomized placebo-controlled trial, results showed a statistically significant decrease in the number (56%) and size (65%) of polyps during the 9 month treatment period compared to the progression of polyposis in the placebo group (70% increase in polyp number and 10% increase in size). It is important to emphasize that the NSAID effect was transient, with regrowth of adenomas in virtually all patients following termination of therapy [14,15].

The reduction in number and size of colorectal polyps was recently confirmed by two international multi-center randomized placebo-controlled trials. One evaluated the effect of Aptosyn™ (Exisulind, sulindac sulfone, Cell Pathway Inc., Horsham, PA, USA) in FAP patients after subtotal colectomy. In this study Aptosyn™ prevented recurrence in 50% of polyps (Arber, personal communication). In the second study a selective cyclooxygenase-2 inhibitor (celecoxib, Searle-Monsanto, Skokie, IL, USA) was used in FAP patients with intact colon, and regression was noted in 35% of the polyps [N. Arber, personal communication].

Epidemiological studies

Overall, 22 of 24 epidemiological studies comprising approximately 20,000 individuals have shown that regular use of aspirin or NSAIDs lowers the risk for colorectal cancer by 40–50% [for reviews see 6–10,16]. The studies were conducted in a variety of settings, using occurrence of cancer or mortality as the primary endpoint. The protective effect was seen in men and women of all age groups. Only one trial showed a null effect [8] while another found an increase in deaths due to colorectal cancer among regular aspirin users [10].

Retrospective prevention studies

Nine retrospective studies in the last decade demonstrated a protective effect of NSAIDs against colorectal cancer [Figure 1]. The relative risk for colorectal cancer among NSAID users ranged from 0.31 to 0.65 [for reviews see 9,16–20].

Kune et al. in 1988 [20] reported the first population-based case-control study. The RR was 0.53 among 715 people who consumed aspirin on a regular basis as compared to 727 non-users. Recently Smalley et al. [9] reported similar results: RR=0.49 among 104,217 non-aspirin NSAIDs users as compared to controls. This study confirmed previous reports and emphasized three important points. The first is that the duration of use and not the dosage is important since low doses of NSAIDs appeared to be at least as effective as higher doses. The second is that most members of the NSAIDs group carry this protective effect; and the third point is that the chemopreventive effect was most pronounced in the right colon. Studies using other anti-inflammatory agents, such as acetaminophen or steroids, did not show any chemopreventive benefit [16].

Prospective studies

Eight of ten prospective studies have demonstrated the protective effect of NSAIDs [Figure 2], although only two were designed to specifically assess the effect of NSAIDs on colon cancer. In most of the studies the history of exposure

FAP = familial adenomatous polyposis
Min = multiple intestinal neoplasia

RR = relative risk

to NSAIDs in patients and controls was determined during the interview at entry to the study. Two studies using acetaminophen, an important confounder, were unable to find any protective effect [6,20].

The most cited study was conducted by the American Cancer Society [21]. In this landmark study one million people were interviewed regarding their personal health habits and cancer risks. The death certificates revealed that of more than half a million individuals, 507 died from colorectal cancer. The relative risk for having colorectal cancer ranged between 0.48 and 0.68 with a correlation to the quantity of aspirin consumed. The greatest reduction in mortality occurred among people consuming more than 16 tablets a month.

The Male Health Professionals study [7] was initiated in 1986 by a mailed questionnaire and included 47,900 non-physician health care workers. The data, confirmed by follow-up in 1988, 1990 and 1992, identified 251 cases of colorectal cancer. Multivariate analysis revealed a marked decrease in colorectal cancer and adenoma risk (RR=0.35–0.68). The protective effect was dependent on the dosage and duration of consumption of NSAIDs.

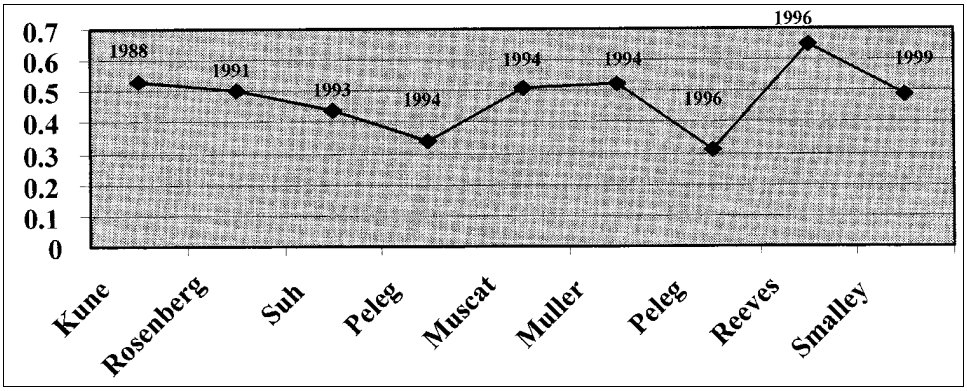

Figure 1. Summary of retrospective studies of NSAIDs usage and colorectal cancer

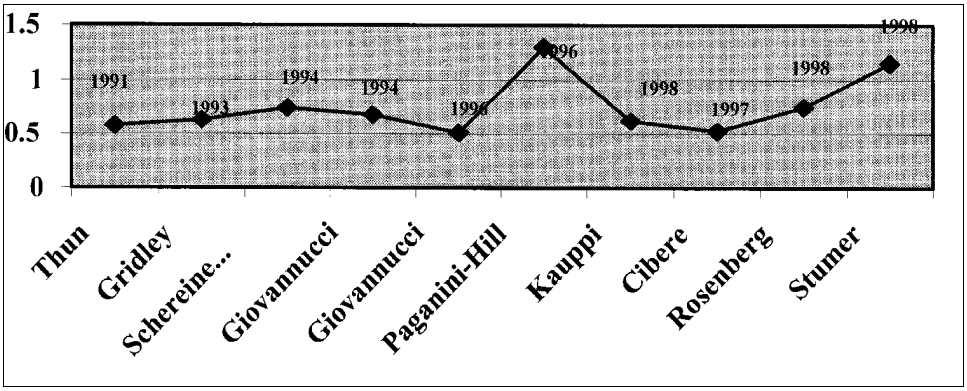

Figure 2. Summary of prospective studies of NSAID usage and colorectal cancer

In 1976, a total of 121,701 female nurses who returned a mailed questionnaire constituted the Nurses' Health study cohort. Giovannucci et al. [6] reported that regular aspirin use substantially reduced the risk of colorectal cancer in this group of nurses. However, the benefit became evident only after a decade of regular use of at least two tablets per week.

Rosenberg et al. [22] collected data from 1,201 patients with colorectal cancer and 1,201 matched controls and confirmed that aspirin and other NSAIDs are equally effective. The regular use of aspirin or NSAIDs until one year before the diagnosis was associated with a significant reduction, 30–40%, in the incidence of colorectal cancer.

However, two studies demonstrated conflicting results. The first, designed to study osteoporosis in 14,000 elderly residents of Southern California, contradicted the protective effects of NSAIDs with the unexpected finding that aspirin use in fact increased the risk for colorectal cancer (RR=1.5) [8]. In a subsequent follow-up 3 years later, still no protective effect from aspirin consumption was seen [8]. This study differed from most other epidemiological surveys in several aspects. The subjects were quite elderly (median age 73) and many of them were health conscious. Additionally, there may have been a bias in ascertaining aspirin usage since the data were based on a single questionnaire session held prior to entry to the study, and therefore non-users might have become users at a later stage.

The second was the Physicians Health Study [10], a large intervention trial to prevent cardiovascular mortality. In this well-designed double-blind trial, 22,071 U.S. physicians were randomly assigned to four groups: placebo, placebo and aspirin (325 mg aspirin every other day), placebo and β-carotene, or β-carotene and aspirin. After 4 years the study was halted earlier than planned because of the clear cardiovascular protection of aspirin. A lower RR (0.86) for polyps and higher RR (1.15) for colorectal cancer were noted. A total number of 33 colorectal cancer cases hampered statistical analysis. The aspirin intervention in this study was fairly short term and at a low dose since its primary endpoint was cardiovascular mortality and not tumor protection. However, even after increasing the follow-up to 12 years there was still no protective effect among aspirin consumers [10].

Colorectal polyps [Figure 3]

Adenomatous polyps are the pre-malignant precursor lesions in colorectal cancer. Since adenoma-free patients are generally kept cancer free, as was shown in the National Polyp Study [4], adenomas can serve as an intermediate biomarker for protection against colorectal cancer.

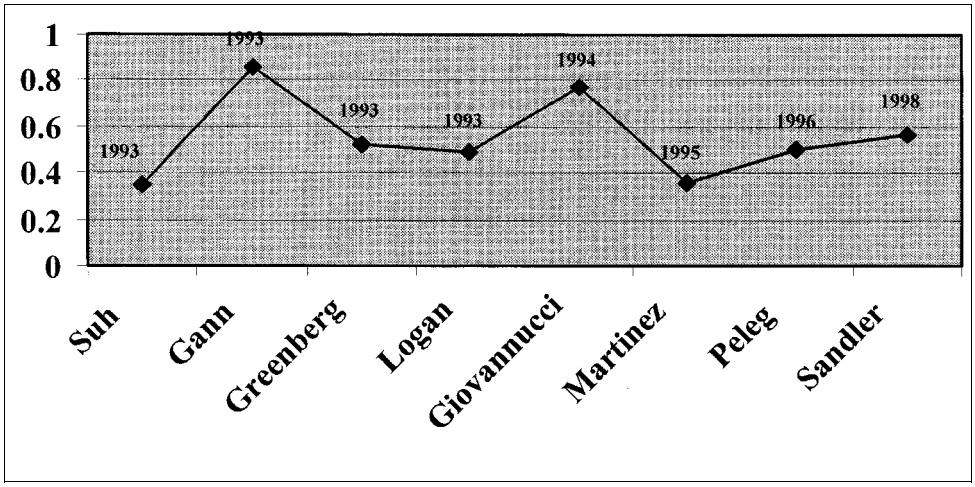

Figure 3. Summary of studies of NSAID usage and colorectal polyps

Compared with FAP, intervention trials in sporadic colonic adenomas are more difficult to perform. The preventive effect in sporadic cases, if present, is much less dramatic than in subjects with FAP. It remains to be clarified whether this is due to the greater difficulty in conducting the trials or whether there is a fundamental difference in the process of colonic carcinogenesis in these two settings.

Eight trials in NSAID users with adenomatous polyps reported a RR of 0.35–0.86 as compared with non-users [19,23–26]. In a case-control study of fecal occult blood screening for colorectal cancer, Logan et al. [25] identified 147 new cases of colorectal polyps in the positive fecal occult blood test. Individuals with a positive and negative fecal occult blood test, but without colorectal polyps, served as controls. The RR for adenoma was 0.6 in aspirin users.

Recently, Sandler and colleagues [20] evaluated the effect of aspirin and NSAIDs in a colonoscopy-based case-control study of 210 patients with and 169 patients without adenoma. After adjusting for potential confounding factors, not only were aspirin users about 50% less likely to develop adenomas but the protective effects lasted at least one year after discontinuation of the drug treatment.

Similar NSAID polyp regression studies have been completed [24–26]. Enrolled were eligible subjects with small polyps in the left colon; the polyps were identified, described, measured, tattooed and left in place. Subjects were then treated with NSAID or, in the case of a controlled trial, placebo. Only one placebo-controlled study, conducted by Landenheim et al. [26], reported no dramatic reduction in the number or size of polyps following 4 months of sulindac therapy. These studies lent support to the hypothesis that NSAIDs function at an early stage in the multi-step process of gastrointestinal tumorigenesis, preceding adenoma formation. It was also suggested that NSAIDs are more effective in causing the regression of right-sided than left-sided colonic adenomas.

A large international multi-center placebo-controlled trial evaluating the effect of exisulind in the regression of sporadic colorectal polyps in the left colon has just been completed. The study code is not yet broken, but polyp regression was noted in some of the patients (Arber, personal experience).

Extracolonic neoplasia in FAP patients

An increasing body of evidence suggests that sulindac and NSAIDs also affect extracolonic tumors, namely duodenal, esophageal and desmoid tumors.

- ***Duodenal tumors:*** These tumors, particularly the periampullary type, are prevalent in FAP patients. Duodenal adnenomas (22–64%) are the putative precursor lesions to duodenal adenocarcinoma. Several controlled trials have reported a decrease in the number and size of duodenal adenomas, and a reduction in proliferation markers in patients on sulindac treatment as compared to the placebo control group [for review see 27]. Similar findings were seen in the recent study performed by the Searle-Monsanto group using celecoxib (the data were not significant), a specific COX-2 inhibitor (Arber, personal communication).
- ***Esophageal tumors:*** The risk of esophageal cancer has been shown by several experimental and epidemiological studies to be reduced by NSAIDs. *In vitro* studies found that NSAIDs (selective and non-selective COX-2 inhibitors) inhibit proliferation of gastrointestinal and esophageal neoplastic cell lines overexpressing COX-2 but do not influence cell lines with low COX-2 levels. While animal studies demonstrated that aspirin and NSAIDs inhibited chemically induced esophageal cancer in rats, there are no interventional data in humans. Nonetheless, epidemiological studies suggest a protective effect among NSAID users [for review see 28].
- ***Desmoid tumors (fibromatoses):*** These tumors may develop sporadically or as part of the FAP syndrome. NSAIDs and in particular sulindac affected both types of desmoid tumors, with a response rate of 40–58% in a relatively small number of patients [for reviews see 15,29,30].

The putative chemoprevention mechanism of NSAIDs

The observation that certain tumors, including colorectal, produce larger amounts of prostaglandins (PGE_2) than the normal mucosa was first reported 25 years ago. Many other groups subsequently confirmed this finding in experimental animals and in humans [for reviews see 30–32]. Their

COX = cyclooxygenase

hypothesis was that the overproduction of PGE_2 might promote the tumor's own growth and spread. PGs are mainly produced by the COX enzymes [30–32], also called prostaglandin-H-synthase. Although the mechanism is still not completely clear [for reviews see 30–37], we now know that the protective mechanism of NSAIDs occurs through several pathways: inhibition of proliferation, induction of apoptosis, prevention of pro-carcinogen activation, and augmentation of the immune response.

• ***Inhibition of proliferation:*** Arachidonic acid can be metabolized into two separate cascades, prostaglandins or leukotrienes, which are catalyzed by different enzymes. In the PG pathway, the COX enzyme has two distinct catalytic activities: in addition to cyclizing and oxygenating arachidonic acid to PGG_2, it also has peroxidase activity whereby it reduces PGG_2 to PGH_2, which can be converted to other PGs or to thromboxanes. There are at least two isoforms of the COX protein encoded by different genes (the gene for COX-1 on chromosome 9 and for COX-2 on chromosome 1). COX-1 is found in the normal gastrointestinal mucosa and is usually constitutively expressed. PGs produced by this enzyme mediate normal physiological function, such as maintaining mucosal integrity and regulating blood flow. Inhibition of COX-1 by NSAIDs is believed to be the cause of NSAID-induced ulcers. COX-2 is undetectable in most normal tissues, but cytokines, growth factors, oncogenes and tumor promoters induce its expression. This enzyme contributes to the synthesis of PGs in inflammatory and neoplastic processes. It has been shown that COX-2 but not COX-1 levels increase in 40% of adenomas and in up to 85% of tumors in colorectal cancer [32]. The inhibition of COX enzymes by NSAIDs diverts the arachidonic acid cascade from PG synthesis into leukotriene metabolites. NSAIDs also directly inhibit cell proliferation, at least in part by down-regulating *cyclin* D1 expression, cyclin-dependent kinase activity CDK_4, and increasing the level of the CDK inhibitor $p21^{waf1}$ [33].

• ***Induction of apoptosis:*** Cell mass homeostasis is a balance between cell proliferation on one hand and apoptosis (programmed cell death) on the other. Inhibition of apoptosis is a vital mechanism in the multi-step development process of colorectal cancer. Recent studies indicate that growth inhibition of colorectal cancer by NSAIDs does not occur necessarily through the inhibition of proliferation alone, but through the induction of apoptosis as well [31–34]. Two evidences support this mechanism. The first was the finding that NSAIDs inhibit cell proliferation in colon cancer cells that do not produce PGs or express COX enzymes [36]; and the second was that Aptosyn™, a metabolite of sulindac (see below) that does not affect COX activity, can inhibit cell growth by induction of apoptosis [35]. Indeed, our group has shown that NSAIDs inhibit proliferation by down-regulating the expression of cyclin D1 protein and inhibiting CDK_4 activity [34]. At the same time NSAIDs increase cell destruction by up-regulating the level of the pro-apoptotic gene *bak* [34].

• ***Prevention of pro-carcinogen activation:*** COX enzymes, and in particular COX-2, are known to metabolize many pro-carcinogenes by their peroxidase activity or through the peroxyl radicals generated during arachidonic acid oxygenation [35].

• ***Augmentation of the immune response:*** Chronic inflammation is a recognized risk factor for epithelial carcinogenesis. Since inflammation per se is associated with increased levels of PGs and COX-2, the overexpression of COX-2 may provide a basis for the link between chronic inflammation and carcinogenesis. The growth of tumors is associated with immune suppression that might be attenuated by NSAIDs, and PGE_2 reduces the expression of HLA I and II antigens [32,36]. In view of the fact that PGE_2 also suppresses T cell proliferation, lymphokine production, macrophage activation, and T cell-mediated cytotoxicity [31,35], NSAID treatment can indirectly augment immune surveillance.

Other possible mechanisms include interference with G protein signal transduction and the trans-membrane calcium influx, as well as the inhibition of other enzymes such as phosphodiesterase, folate-dependent enzymes and cyclic AMP. It was also suggested that NSAIDs induce terminal differentiation and inhibit reactive oxygen radicals [31,35].

Specific inhibition of COX-2

This review demonstrates that NSAIDs can prevent colorectal cancer. But at what price? It is well known that these drugs are extremely toxic to the renal and intestinal mucosa. In fact, in the United States in 1998 there were 107,000 hospitalizations and about 50 patients died every day as a consequence of using this class of agents.

Data from several studies suggest that inhibition of PG synthesis, particularly through inhibition of COX-2, can be chemopreventive [31,32,35]. COX-1 is found in normal gastriointestinal mucosa and its inhibition by NSAIDs is thought to be the mechanism of NSAID-induced ulcers [33,34]. It was shown that COX-2 overexpression in an intestinal epithelial cell line (RIE-1) blunts the apoptotic effects of NSAIDs [33]. Our findings were similar [34] in normal enterocytes transformed with a c-K-*ras*. Sheng's group [36] demonstrated that a selective inhibition of COX-2 inhibited colon cancer cell growth, and Reddy et al. [30] showed that a selective COX-2 inhibitor exerted chemopreventive activity in the rat aberrant crypt focus model induced with azoxymethane. Most recently, by crossing COX-2 knockout mice with APC mutant Min-mice, Oshima et al. [12] noted a marked reduction in the number of intestinal adenomas. This study directly demonstrates that regulation of COX-2 appears to affect colonic carcinogenesis.

COX-2 is not usually detectable in the normal GI mucosa [37]. Up-regulation of COX-2 expression occurs in 40–50% of colorectal polyps and in the majority of neoplasia tumors

PGs = prostaglandins
PGH = prostaglandin-H-synthase
CDK = cyclin-dependent kinase

[30,37]. The lack of COX-2 expression in the normal colonic mucosa and its increased expression in colonic neoplasm is the rationale for a selective action of COX-2 inhibitors on neoplastic colon mucosa, without major biologic effects on the normal colonic mucosa or the risk of gastroduodenal ulcers. COX-2 enzyme is therefore a potential target for the biochemical chemopreventive actions of NSAIDs.

The expression of COX-2 in stimulated colon cell lines was shown to occur predominantly in a peri-nuclear distribution, suggesting a possible mechanism of action through a nuclear receptor. A potential candidate is the peroxisome proliferator-activated nuclear receptor (PPARδ) family, whose expression is closely linked with that of the retinoid receptor family. In two recent studies [38,39], PPARδ was identified as a target for regulation by the *APC* tumor suppressor gene. The increased expression of PPARδ in colorectal cancer cells was repressed by the *APC* gene. This repression was mediated by β-catenin/Tcf-4-responsive elements in the PPARδ promoter. It was also shown that sulindac repressed PPARδ-responsive elements and was able to disrupt the binding ability of PPARδ to its recognition sequences. These reports suggest that NSAIDs inhibit colorectal tumorigenesis through repression of PPARδ, which is normally regulated by the *APC* tumor suppressor gene.

COX-2-specific inhibitors are now commercially available. They offer all the well-known benefits of aspirin or NSAIDs, namely relief of pain, fever and inflammation, but without gastric toxicity. These models may be particularly relevant for the chemoprevention of sporadic colorectal cancer since aberrant crypt foci are recognized as early preneoplastic lesions in the colonic mucosa of these patients. Their results might be noteworthy in that the degree of inhibition of colon carcinogenesis exceeded those with other commonly used NSAIDs [30]. Moreover, long-term administration of celecoxib at 1,500 ppm did not induce any toxic side effects. The same group also showed that increased expression of COX-2 is an early event in the sequence of polyp formation, and that celecoxib inhibits initiation as well as promotion and progression phases of colorectal carcinogenesis [30].

The utility of selective COX-2 inhibitors as chemopreventive agents is currently under investigation. A controlled trial of Celebrex (Searle-Monsanto Inc, Skokie, IL, USA) in FAP patients with intact colon was completed last year. Eighty-one patients from London (St Mark's Hospital) and Texas (MD Anderson Medical Center) were randomly selected to receive two dosages of the drug (400 or 800 mg) or a placebo for 6 months. At the end of the study, polyp burden (size and number) was reduced by 30–40% [Arber, personal communication]. In 1999, Searle Monsanto launched an international multi-center study to evaluate the efficacy of their new specific COX-2 inhibitor (celecoxib) in preventing the recurrence of sporadic colorectal polyps (Arber, personal communication). Merck Sharp and Doehm (Rahway, NJ, USA) will also study its COX-2 inhibitor (rofecoxib) in a similar trial starting this year.

COX-2 is not the entire story: Is SAAND the ultimate drug?

It seems likely that inhibition of PG synthesis is but one biochemical target for NSAID action. Several lines of evidence suggest that there are biochemical targets other than COX-2 that mediate the chemopreventive activity of NSAID-type drugs [20,33,35,36].

Sulindac is a pro-drug (a sulfoxide) that rapidly metabolizes in colonocytes and hepatocytes [34]. About half of the sulfoxide is initially converted by a reversible oxidation/reduction reaction to sulindac sulfide, which inhibits both COX-1 and 2 and is therefore a potent anti-inflammatory drug. The other half of the sulfoxide is inversely reduced to a sulfone metabolite. Exisulind (Aptosyn™ sulindac sulfone) is not an NSAID since it lacks anti-inflammatory properties and does not inhibit COX-1 or COX-2 [34]. Due to the reversibility of the sulfide reaction, the sulfone is ultimately the major sulindac metabolite in humans. Aptosyn™ is a selective apoptotic anti-neoplastic drug. As an inhibitor of PG synthesis *in vitro* or in the rat colon it is at least 5,000-fold less potent than the sulfide metabolite, yet prevented carcinogen-induced cancers in the AOM rat colon cancer model [30,34,35]. Recently, the underlying mechanism of the drug was revealed [30]. In this novel mechanism the inhibition of cyclic GMP phosphodiesterase activity is selective only in neoplastic tissue and not in normal mucosa.

The basal apoptotic rate is significantly lower in adenomas from FAP patients than in sporadic adenomas, and Aptosyn™ treatment increases the apoptotic rate threefold without affecting the rate of proliferation. Similar preliminary results were obtained in a clinical trial of Aptosyn™ in FAP subjects [40]. A Phase II clinical trial of Aptosyn™ in human subjects with FAP demonstrated that the drug resulted in more than a 50% reduction in colonic adenomas. The most effective dose with the least number of side effects was 150 mg qid [40]. In a randomized international multi-center placebo-controlled trial in the USA, UK, Sweden and Israel completed last year, 74 patients received sulindac sulfone (150 mg qid) or placebo for 12 months. The primary endpoint was the number of polypectomies. At the end of the study there was an approximately 50% reduction in polyp number recurrence in the treatment group (Arber, personal communication); and when all placebo patients, regardless of subgroup, were crossed-over to the drug a 50% reduction in the polyp formation rate within 6 months was observed ($P=0.005$). All patients from the drug-treated group who continued the regimen for an additional 6 months showed a 58% additional reduction in their polyp formation rate ($P=0.006$). This endorses the Phase III findings and confirms that the patients continue to get better without losing the drug effect for up to 18 months.

PPAR = peroxisome proliferator-activated nuclear receptor

SAAND = selective apoptotic anti-neoplastic drug

Summary

Perhaps the most important consequence of Waddell and Loughry's sentinel observations [13] is the regression of polyps in FAP patients taking sulindac. It has become a model for the investigation of the biological and biochemical mechanisms of chemoprevention using a class of agents that have demonstrable activity in human neoplastic tissue. We have only just begun to unravel the mechanisms of cancer chemoprevention; without doubt there is much more to come. It is very likely that more effective chemopreventive agents will be designed in the future that will be based on these new discoveries. Our challenge is to find the proper place for chemoprevention in the entire effort of cancer prevention, not only in subjects at risk for colon cancer but those at risk for other cancers as well.

Taken together, current evidence strongly indicates that the NSAID class of drugs can inhibit the process of colonic carcinogenesis effectively and safely. However, in order to offer optimal protection several issues have yet to be clarified. An intriguing mystery is whether there is one, two or more biochemical targets for the chemopreventive effects of NSAIDs and SAANDs. The presence of multiple potential biochemical targets is potentially very good news, with the possibility that combinations of potent inhibitor targets could be more effective than either agent alone. One or more of the NSAID targets may have an even greater role to play in cancer sites that are less amenable to prevention, screening and surveillance programs than colorectal cancer.

We believe that the pivotal question as we enter the third millennium is not if but how? There is no need for further placebo trials, but additional intervention trials are necessary to resolve several issues before a definite recommendation can be made for the widespread use of NSAIDs to prevent colorectal cancer. What is the ultimate drug (SAAND or specific COX-2 inhibitor)? What is the optimal dose? What is the optimal age to initiate chemoprevention? What is the optimal duration of therapy? How frequently should surveillance be performed? At the same time, we must remember that the standard of care for patients with colorectal adenomas is still polypectomy and not chemoprevention.

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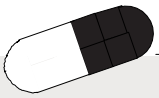
Capsule



Scrapie strategy

Scrapie is carried by an abnormally folded, infective form of the prion protein, which then directly induces the abnormal form in previously normal prion protein molecules to cause disease in sheep, mice and other animals. When animals are infected, the abnormal prions replicate in the spleen and then move into the central nervous system, a process that requires an intact immune system. Montrasio et al. have now shown that dendritic cells are the key cellular element necessary for prion replication and neuro-invasion. They eliminated follicular dendritic cells, an antigen-presenting cell in the spleen, by injecting a soluble lymphotoxin-beta receptor that inhibited formation of functional dendritic cells. The similarity between the clinical course of scrapie and vCJD (the new variant of Jacob-Creutzfeld disease seen in humans and possibly derived from bovine spongiform encephalopathy) suggests that interfering with the lymphotoxin-beta receptor system may also delay the progress of the human disease.

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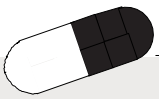
Capsule



When did HIV arise?

An analysis of nucleic acids can reveal the time when an organism appeared during history. Korber et al. analyzed HIV-1 sequences to estimate the timing of the ancestral sequence of the main group of HIV-1, the strains responsible for the AIDS pandemic. Using parallel supercomputers and assuming a constant rate of evolution, they applied maximum-likelihood phylogenetic methods to unprecedented amounts of data for this calculation. They validated their approach by correctly estimating the timing of two historically documented points. Using a comprehensive full-length envelope sequence alignment, they estimated the date of the last common ancestor of the main group of HIV-1 to be 1931 (1915–41). Analysis of a gag gene alignment, subregions of envelope including additional sequences, and a method that relaxed the assumption of a strict molecular clock also supported these results.

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