*The Fourth DeWitt S. Goodman Lecture*

# Novel Approaches to the Prevention of Colon Cancer by Nutritional Manipulation and Chemoprevention[1]

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## Abstract

**Large bowel cancer is one of the most common human malignancies in Western countries including North America. This report details the preventive strategies aimed at reducing the incidence and mortality of large bowel cancer by nutritional manipulation and chemopreventive agents. During recent decades, multidisciplinary research in epidemiology and laboratory animal model studies have contributed much to our understanding of the etiology of this cancer; more importantly, it has enabled us to approach cancer prevention. An impressive body of data thus far accumulated has provided important concepts about dietary factors such as fat and fiber as key modulators of large bowel cancer. Compelling experimental evidence indicates that certain dietary lipids and fibers influence tumorigenesis in the colon. Data obtained in metabolic epidemiological and laboratory animal model studies are sufficiently convincing in showing the enhancement of colon cancer by certain types of fat and protection against it by certain dietary fibers. Our approach to the primary prevention of large bowel cancer is to translate the findings from clinical epidemiological and laboratory studies into sound advice for patients and for the public at large to reduce fat intake and increase fiber intake, specifically cereals and grains. Preclinical efficacy studies have provided scientifically sound evidence as to how several phytochemicals and their synthetic analogues act to retard, block, or reverse carcinogenesis. Equally exciting are opportunities for effective chemoprevention with nonsteroidal anti-inflammatory agents, both synthetic and naturally occurring, or selective cyclooxygenase-2 inhibitors. Our exploration of the multistep process of carcinogenesis has provided substantial insights into the mechanisms by which chemopreventive agents modulate these events. Growing knowledge in this area has brought about an innovative combination of agents with different modes of action as a means of increasing efficacy and minimizing toxicity. There is growing optimism for the view that realization of preventive concepts in large bowel cancer will also serve as a model for preventing malignancies such as cancer of the prostate and breast.**

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## Introduction

As I reflect on my research activities in colon cancer prevention since 1971, I recognize the contributions of several colleagues and collaborators at the American Health Foundation and worldwide who have had a major impact on my scientific achievements. I thank them for their continued support. This lecture summarizes our approaches to the reduction of the incidence and mortality of colorectal cancer by nutritional manipulation and chemopreventive agents.

Large bowel cancer is one of the most common and persistent human malignancies in the Western world, including the United States. Globally >875,000 men and women were afflicted with this cancer in 1996. More than 510,000 individuals died of colon cancer in 1996 (1). The American Cancer Society estimates that in the United Sates, there will be 137,000 new cases and about 57,000 deaths attributable to this cancer alone in 1999; thus, large bowel cancer is a major public health problem (2). An impressive body of evidence supports the concept that dietary factors are key modulators of colorectal cancer. Prevention strategies that embrace intervention by nutritional modification and chemopreventive agents that can retard, block, or reverse the process of carcinogenesis or reduction of the underlying risk factors can be applied across a continuum of the general population. Such application is urgent for persons with precancerous lesions, those diagnosed at early stages, and for subgroups with particular genetic susceptibility to cancer.

Chemoprevention has the potential to be a major component of colorectal cancer control. Several investigators have over many years conducted research on agents with potential chemopreventive properties and have elucidated their modes of action. Although full explanation of the intricacies of the causes, development, and control of colon cancer is awaiting further research, the growing knowledge about mechanisms by which chemopreventive agents act defines opportunities to use specific agents or combinations of them at critical stages of cancer initiation, promotion, and progression. Progress in the area of chemoprevention during the past two decades has been very impressive. Although the scientific community has recognized the potential contribution of chemoprevention to colorectal cancer prevention and control, the optimal approach for the prevention of this cancer is seen in a combination of nutritional manipulation with the application of chemopreventive agents.

## Nutritional Factors and Colon Cancer

There is overwhelming epidemiological and experimental evidence that dietary factors are the most important determinant of

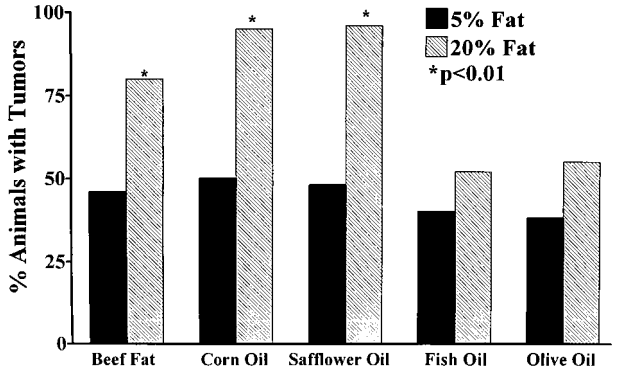


*Fig. 1.* Amount and types of dietary fat on azoxymethane-induced colon carcinogenesis in F344 rats (4).

colon cancer development. In Western countries, the risk attributable to diet and related nutritional factors has been estimated to be ~50%. Although epidemiological studies indicate important roles for energy balance (body mass and exercise), cooking of meat, total fat, especially saturated fat, and fiber, the discussion on nutritional factors and colon cancer risk will be limited to dietary fat and fiber and colon cancer.

### Dietary Fat and Colon Cancer

Diet, especially fat intake, has long been regarded as the most important nutritional influence on colon cancer development. On the basis of comparative data and case-control studies in Japan and the United States in the late 1960s, Wynder *et al.* (3) proposed that colon cancer incidence is mainly associated with total dietary fat intake and, that furthermore, dietary fat influences the composition of the gut microflora, which is likely to be involved in the pathogenesis of colon cancer. This pioneering investigation led to several ecological and case-control studies on the relationship between total dietary fat and the development of colon cancer in humans. The conduct and interpretation of some of these studies has been complicated by problems inherent in testing the dietary hypothesis. The reliability, validity, and sensitivity of the hypotheses to reveal narrow but biologically significant differences and factors of dose standardization have been questioned. For example, a major difficulty with several earlier studies has been the lack of an accurate measurement of the types of fat, which differ in fatty acid composition; after all, laboratory animal model studies had by then provided evidence that the colon tumor-promoting effect of dietary fat depends on the type of fat (4). Evidence for the association between saturated fat and/or animal fat intake and colon cancer risk is strong (5). Several case-control and ecological studies on the relationship between dietary saturated and/or animal fat consumption and colon cancer risk found that higher intakes of these types of fat increase the risk of colon cancer, with odd ratios ranging from 1.5 to 2.6 (5). On the other hand, diets rich in polyunsaturated fats with $\omega$-3 fatty acids such as those in fish and fish oil were hypothesized to decrease the risk of colon cancer (6).

Laboratory animal model assays have provided unequivocal evidence that intake of high amounts of saturated fats, such as lard, beef tallow, and polyunsaturated fats, such as corn oil and safflower oil, increase the risk of chemically induced colon carcinogenesis (Ref. 4; Fig. 1). By contrast, diets high in olive oil and fish oil had no such colon tumor-promoting effect (4). Corn oil and safflower oil are very rich in $\omega$-6 fatty acids (LA[3]), olive oil is rich in monounsaturated fatty acids (oleic acid), and fish oil is high in $\omega$-6 fatty acids (DHA and EPA). The varied effects of different types of fat on colon carcinogenesis suggests that fatty acid composition is one of the determining factors in colon tumor promotion (4). In this regard, it is noteworthy that diets high in beef tallow, lard, or corn oil increase the concentration of colonic luminal (fecal) secondary bile acids, *i.e.*, deoxycholic acid and lithocholic acid (4), whereas high dietary fish oil had no such enhancing effect. Model assays in laboratory animals have demonstrated that these secondary bile acids induce cell proliferation and act as promoters in colon carcinogenesis (7).

Metabolic epidemiology studies have shown that populations at high risk for colon cancer excrete high levels of secondary bile acids (8). Individuals at increased risk for colon cancer have also been found to have abnormal patterns of cell proliferation, including higher rates of DNA synthesis in normal-appearing colorectal mucosa (9). These changes precede tumor development and, therefore, constitute a key step in colon carcinogenesis (9). It is important to note that high concentrations of luminal secondary bile salts also increase cell proliferation in the colonic epithelium (9).

Another mechanism by which high dietary fat intake, specifically saturated fat and polyunsaturated fat rich in $\omega$-6 fatty acids, increases colon tumor promotion is through the alteration of membrane phospholipid turnover and PG synthesis. This became evident from the observation that secondary bile salts stimulate the membrane phospholipid turnover through the activation of phospholipases, especially $A_2$ (10). Also, DHA and EPA from fish oil can partially replace AA and LA in the phospholipid pool and modulate the activity of $PLA_2$ and phosphatidylinositol-specific phospholipase C involved in the release of fatty acids from phospholipids (11). As a result of increased phospholipid turnover and the release of free AA and other products of phospholipid breakdown, several biologically active compounds that are generated locally in the colon may alter cellular proliferation (12). In support of this, there are studies showing that $PLA_2$ activity is significantly higher in colonic tumors than in the normal colonic mucosa of rats (13). Also, a high-fat diet containing corn oil increased colonic mucosal and tumor $PLA_2$ as well as phosphatidylinositol-specific phospholipase C as compared with low doses of dietary corn oil or a diet high in fish oil (10, 13). Importantly, increasing levels of fish oil in the diet raised the $\omega$-3 fatty acids, *i.e.*, DHA and EPA, in phospholipids at the expense of the $\omega$-6 fatty acids such as LA and AA (14). Only after liberation from membrane phospholipid is AA available for further enzymatic modification by COX, LOX, and monooxygenases. AA released from phospholipids is metabolized via COX to a number of prostanoids. It is of great interest that the $\omega$-3 fatty acids in fish oil, *i.e.*, DHA and EPA, inhibit the COX pathway as well as AA metabolism for PG synthesis (15). Consistent with this, there are studies demonstrating that diets rich in $\omega$-6 fatty acids increase overall COX activity in colonic mucosa and tumors during the promotion and progression stage, whereas diets low in $\omega$-6 or high in $\omega$-3 fatty acids have no such impact (13, 16).

[3] The abbreviations used are: LA, linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PG, prostaglandin; AA, arachidonic acid; $PLA_2$, phospholipase $A_2$; AOM, azoxymethane; BSC, benzylselenocyanate; COX, cyclooxygenase; DFMO, difluoromethylornithine; ODC, ornithine decarboxylase; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; NSAID, nonsteroidal anti-inflammatory drug; FAP, familial adenomatous polyposis; *p*-XSC, 1,4-phenylenebis(methylene)selenocyanate.

Importantly, at least two COX isozymes have been identified; of these, *COX-1* is thought to be a constitutively expressed gene, whereas *COX-2* is induced by cytokines, growth factors, and tumor promoters (17). Also, mitogen-inducible *COX-2* expression is up-regulated in human colorectal carcinomas. In addition, intestinal epithelial cells overexpressing the *COX-2* gene develop altered adhesion properties, and they resist undergoing apoptosis (18). Therefore, overexpression of COX-2 may alter the tumorigenic potential of intestinal epithelial cells. A markedly elevated expression of *COX-2* but not *COX-1* gene expression has been observed in colonic mucosa and tumors of rats induced with carcinogens (19). Also, decreased expression of *COX-2* has been found in the colonic mucosa and tumors of rats in carcinogenesis assays when they were fed a low-fat corn oil diet or high-fat fish oil diet as compared with a high-corn oil diet (16).

## Dietary Fiber and Colon Cancer

The hypothesis that a diet high in fiber may protect against colon cancer was first proposed by Burkitt (20), who observed that African Blacks consuming foods with high fiber but low-fat content had lower death rates from colon cancer than did their white counterparts, who ate low-fiber, high-fat diets. Subsequent studies demonstrated that populations eating diets high in total fat, especially saturated fat but at the same time, diets high in total fiber, fibrous foods, and certain whole-grain foods, had a reduced risk for colon cancer (21, 22). Intra-country comparisons of dietary fiber intake and colon cancer mortality rates suggest that dietary fiber, especially fiber from cereal sources and pulses, protects against colon cancer (23). We have explored the dietary patterns and fecal bile acid levels in relation to the risk of colon cancer in Scandinavian and Finnish populations, who had varying risks for colon cancer development (22). One of the factors apparently associated with the low risk of colon cancer in certain areas of Finland is a high intake of dietary fiber, averaging 35 g/day, mainly 17–21 g/day of cereal fiber; in comparison, New Yorkers had an average total dietary fiber intake of only 12 g/day. This increase in dietary fiber intake in rural Finland is associated with a decrease in fecal secondary bile acids (Fig. 2). A recent prospective study of nurses by Fuchs *et al.* (24) suggests no protective effect of total dietary fiber or dietary fiber from cereals, fruits, or vegetables against colon cancer or adenoma. In this study, total daily fiber intake ranged from 9.8 to 24.9 g, with cereal fiber contributing 1.0–4.8 g and vegetable fiber contributing 2.7–10.9 g. The total fiber intake of the quintile with the highest fiber intake was actually rather low compared with the total fiber intake of the Finnish population in our study. The contrast is especially great with regard to cereal fiber, of which the highest consumption was 4.8 g/day in the nurses' study and 17–21 g/day in our Finnish study. It is important to study the effects of specific kinds of dietary fiber rather than treating a mixture of heterogeneous fibers as a single variable. Howe *et al.* (25) performed a meta-analysis of 13 case-control studies conducted in populations with differing colon cancer rates and dietary practices. In 12 of the 13 studies, the relative risk decreased significantly as fiber intake increased. Relative risks were 0.79, 0.69, 0.63, and 0.53 for the four highest quintiles of intake compared with the lowest quintile, with risk reduction of almost 50% between the lowest and the highest quintiles of fiber intake. Similar findings have been reported for a meta-analysis of 16 case-control studies with an odds ratio of 0.6 for highest *versus* lowest dietary fiber intake (26).

Our own diet intervention studies in humans indicate that certain types of dietary fiber favorably altered a number of biomarkers in the colon that are related to risk of colon cancer (27, 28). Because of the potential significance of colonic secondary bile acids in the pathogenesis of colon cancer, we have investigated the effect of different types of dietary fiber, *i.e.*, wheat bran, corn bran, or oat bran, on fecal secondary bile acids in healthy males and females. The rationale for selecting these three sources of fiber was based on the fact that they not only differ from each other with respect to composition, fermentability, and solubility but also constitute important sources of grain fiber in the United States and Western countries. The subjects consumed 13–15 g of wheat, corn, or oat bran daily for 8–12 weeks in addition to their Western diet containing about 12 g of dietary fiber. These studies revealed that the dietary wheat bran, but not corn bran or oat bran, decreased the levels of secondary bile acids such as deoxycholic acid and lithocholic acid in stools. The outcomes of fiber studies in laboratory animal assays also suggest that: (*a*) the inhibitory effect of dietary fiber, which comprises heterogeneous groups of nonstarch polysaccharides such as cellulose, hemicelluloses, pectin, and gums, and the noncarbohydrate substance lignin depends on the nature and source of fiber; and (*b*) wheat bran appears to inhibit colon tumor development more consistently than other sources of fiber, such as oat and corn brans (29).

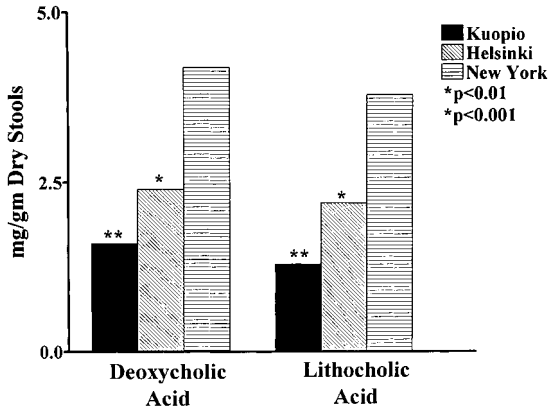


*Fig. 2.* Fecal secondary bile acids in healthy subjects from rural Kuopio and urban Helsinki (Finland) and New York (22).

More recent studies in our laboratory have compared how altering both fiber and fat content in the diet affects fecal secondary bile acids in healthy subjects. Individuals who were consuming a typical high-fat, low-fiber Western diet were switched to a low-fat, low-fiber diet and then to a low-fat, high-fiber diet. Analyses of fecal contents for secondary bile acids showed a dramatic reduction in both deoxycholic acid and lithocholic acid during the low-fat and high fiber period and a moderate reduction in these bile acids during the low-fat, low fiber period, compared with the high-fat and low-fiber period (Fig. 3).

## Chemoprevention of Colon Cancer

The development of strategies for chemoprevention has been markedly facilitated by the use of relevant animal models mimicking the neoplastic process that occurs in humans. The rat model for carcinogen-induced colon cancer has been used in our studies to obtain critical information on the chemopreventive efficacy of several agents. Chemoprevention refers to the administration of chemical agents, those naturally occurring in

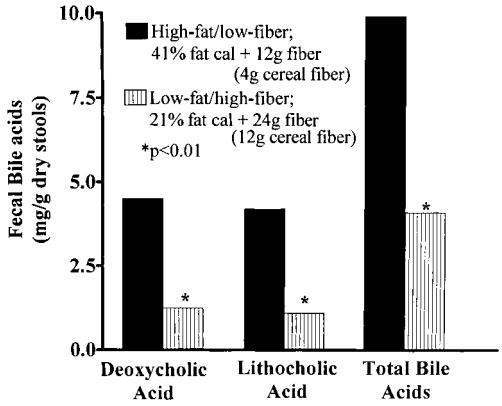


*Fig. 3.* Fecal secondary bile acids in healthy subjects during the high-fat/low-fiber and low-fat/high-fiber dietary regimens.

foods as well as synthetic analogues that may block the tumor initiation (mutation) and promotion events that are the sequential stages of cancer development (30, 31). Inhibition of these processes before the occurrence of clinically detectable tumors is receiving increasing attention as an attractive and plausible approach to cancer control. Growing knowledge of the mechanisms by which chemopreventive agents act defines opportunities to use specific agents at critical points in carcinogenesis. Wattenberg (30) has classified chemopreventive agents into three broad categories, with distinctly different functions: agents that can prevent the formation of carcinogenic compounds from their precursors; agents that can block the metabolic activation of carcinogens; and agents that can suppress the expression of neoplasia in cells exposed previously to an effective dose of carcinogen. In addition, several agents have been shown to inhibit carcinogenesis both by blocking the metabolic activation of carcinogens and by suppressing the promotion and progression of carcinogenesis.

### Phytochemicals as Chemopreventive Agents

Several studies have demonstrated that generous consumption of vegetables reduces the risk of colon cancer (31). Although the nature of the constituents of these vegetables and other food items that are responsible for reduced risk has not been fully elucidated, it is clear that the plant foods contain chemopreventive agents, including several micronutrients, such as vitamins, and minerals and also contain nonnutrient phytochemicals, such as organosulfur compounds, polyphenols, and isoflavones, to cite a few. The diversity of these compounds is a positive feature, indicating that a variety of approaches to cancer prevention by these agents may be made so that the optimal selection will emerge. Our approach to the development of effective chemopreventive agents for the secondary prevention of colon cancer has been to evaluate the inhibitory role of several phytochemicals of dietary origin and their synthetic analogues in a preclinical animal model. Mechanisms of chemopreventive activity of these agents range from inhibition of carcinogen activation to detoxification of the carcinogen, blockage of binding of critical carcinogen metabolites to DNA, scavenging reactive electrophiles, and inhibiting AA metabolism. The recognition that colon cancer progresses through discrete pathological changes from normal mucosa to abnormal hyperproliferative epithelium has aided the development of surrogate intermediate biomarkers (32). The phytochemicals and their substituted and synthetic analogues tested for their efficacy in our animal model assay included anethole trithione, oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione], diallyl sulfide, and curcumin.

Administration of organosulfur compounds such as diallyl sulfide, oltipraz, or anethole trithione during the initiation and/or postinitiation stages significantly suppressed the incidence and multiplicity of AOM-induced colonic adenocarcinomas in male F344 rats (33–35). The inhibition of colon carcinogenesis by these agents was associated with an increase in the activities of detoxifying enzymes such as glutathione *S*-transferase, quinone reductase, and UDP-glutathione transferase in the colonic mucosa and tumors. The protective effect of these compounds may, at least in part, be attributable to their ability to induce the detoxifying phase II enzymes in the colon. It may also be attributable to blocking the formation of ultimate carcinogen electrophiles, perhaps by trapping free radicals involved in such formation. However, the full elucidation of mechanisms of chemopreventive activity of these agents administered during the postinitiation period requires more detailed study.

Curcumin [diferuloylmethane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione)], which has been identified as the major pigment in turmeric, the powdered rhizome of *Curcuma longa Lim*, possesses both anti-inflammatory and antioxidant properties. Importantly, dietary administration of curcumin also reduces formation of focal areas of dysplasia and aberrant crypt foci in the colon, which are early preneoplastic lesions in rodents (36). We have shown that continuous dietary administration of 0.2 and 0.4% curcumin during the initiation and postinitiation stages of AOM-induced colon carcinogenesis significantly inhibited the incidence and multiplicity adenocarcinomas and the total tumor burden in F344 rats (37). We have also found that curcumin, given as a dietary supplement during the promotion/progression period, dramatically inhibited colon tumorigenesis (38). This suggests that curcumin may retard growth and/or development of existing neoplastic lesions in the colon and that this agent may be an effective chemopreventive agent for individuals at high risk for colon cancer development, such as patients with polyps. With regard to its mode of chemopreventive action, curcumin exhibits an array of metabolic, cellular, and molecular activities, including inhibition of AA formation and its further metabolism to eicosanoids. In our assays, dietary administration of curcumin significantly inhibited $PLA_2$ in the colonic mucosa and tumor tissues, leading to the release of AA from phospholipids, and it altered COX activity and modified $PGE_2$ levels (37). Several lines of evidence also indicate that the mechanism of action of curcumin is not limited to PG inhibition. We had observed earlier that dietary curcumin inhibits LOX activity and blocked the production of the LOX metabolites, 5(*S*)-HETE, 8(*S*)-HETE, 12(*S*)-HETE, and 15(*S*)-HETE, in the colonic mucosa and in tumors (37). Importantly, 12(*S*)-HETE is known to promote tumor cell adhesion, to stimulate the spreading of tumor cells, and to augment metastatic potential (39). Curcumin also inhibits several mediators and enzymes involved in the mitogenic signal transduction pathways of the cell and AP-1 and nuclear factor-κB activation (40). Hanif *et al.* (41) provided evidence that curcumin inhibits cell proliferation and induces cell cycle changes in the colonic adenocarcinoma cell lines, HT-29 and HCT-15, and that this effect is independent of the ability of curcumin to inhibit PG synthesis. The inhibitory effect of curcumin when administered during the promotion/progression stage of carcinogenesis is associated with increased apoptosis, suggesting that increased cell death through apoptosis may be

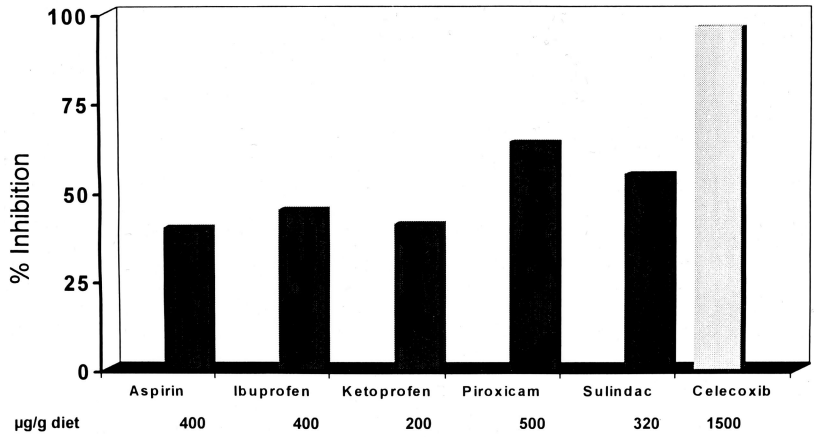


*Fig. 4.* AOM-induced colon tumor inhibition in F344 rats by NSAIDs and celecoxib, a selective COX-2 inhibitor (51–53, 64).

one of the mechanisms by which dietary curcumin blocks the progression of colon carcinogenesis.

### NSAIDs as Chemopreventive Agents

In recent years, attention has been drawn to the potential chemopreventive properties of NSAIDs. An effective preventive strategy for colon cancer could be envisioned on the basis of recent epidemiological, clinical, and laboratory investigations that combine to present an inverse relationship between the use of NSAIDs and colorectal cancer development. These studies have consistently reinforced the evidence that NSAIDs are indeed very effective chemopreventive agents against colon cancer development. The clinical studies suggest therapeutic use of sulindac, an NSAID, in patients with FAP (42). Labayle *et al.* (43) reported that in a randomized, placebo-controlled, double-blind crossover study in patients with FAP, administration of sulindac at a dose of 300 mg/day for 6–12 months caused disappearance of all colonic polyps. In another study, the incidence and size of adenomas were reduced in FAP patients after long-term therapy with sulindac (44). Although the dosage of sulindac administered in these studies varied from 150 to 400 mg/day, most of the patients treated with this drug exhibited full remission, whereas some patients showed a partial response.

Several case-control and cohort studies have provided unequivocal evidence for the inverse relationship between colon cancer and the use of NSAIDs, specifically aspirin (45–47). The first study that examined the relationship between aspirin use and colorectal cancer in Melbourne, Australia, demonstrated a highly significant protective effect in both men and women (45). After this investigation, several other case-control studies likewise reported a protective effect of aspirin against colorectal cancer (46). The results of a large American Cancer Society Cancer Prevention Study of >620,000 people are very consistent in showing a strong inverse relationship between the use of aspirin and colon cancer risk (47). A case-control study of adenomatous colorectal polyps also indicates a significant protective effect of aspirin use and colorectal adenoma formation (48).

There has been ample and consistent experimental evidence from laboratory animal model studies to indicate that NSAIDs, including indomethacin, piroxicam, sulindac, aspirin, ibuprofen, and ketoprofen, inhibit chemically induced colon cancer. Pioneering studies by Narisawa *et al.* (49) and Pollard and Luckert (50) demonstrate that indomethacin and piroxicam, administered to rodents in drinking water, diet, or i.p., inhibited colon tumors induced by a variety of carcinogens. Since then, a number of investigations have evaluated the chemopreventive efficacy of several NSAIDs against colon carcinogenesis (51–54). These studies have demonstrated that administration of NSAIDs including aspirin, ibuprofen, piroxicam, ketoprofen and sulindac during the initiation and postinitiation stages suppressed the incidence and multiplicity of colon tumors (Fig. 4). Of particular interest is that aspirin, piroxicam, and sulindac even reduced spontaneous intestinal tumorigenesis in *APC*$^{min}$ mice, who are genetically predisposed to develop intestinal tumors (55, 56).

The above-cited studies clearly demonstrate the potential chemopreventive activity of NSAIDs against colon carcinogenesis when these agents were administered during the initiation and postinitiation stages of carcinogenesis. However, the multistep nature of carcinogenesis provides many opportunities for intervention with agents targeted at specific mechanisms involved in the initiation, promotion, and progression of cancers. Determining the efficacy of these agents during the promotion and progression stage, at which point premalignant lesions are known to have developed, is very important with regard to the eventual clinical use of these agents in secondary cancer prevention among patients with colonic polyps. We have provided evidence that piroxicam and sulindac administered during the promotion/progression stage are agents that can still significantly inhibit colon tumorigenesis at this stage (52, 53). Results generated in this preclinical model assay provided baseline information for eventual clinical evaluation of the efficacy of NSAIDs in the late intervention/prevention protocols of colonic tumors in high-risk individuals, such as patients with sporadic colonic polyps or FAP. In conclusion, epidemiological, clinical, and laboratory animal model studies together have provided a strong case that the use of NSAIDs is effective in reducing the risk of colon cancer.

The mechanisms by which NSAIDs act to reduce the risk of colon carcinogenesis is not yet clearly understood. Accumulating evidence points to inhibition of AA metabolism via COX enzymes, which in turn modulate the synthesis of PGs that affect cell proliferation, tumor growth, and immune responsiveness (57). Two mammalian isozymes, COX-1 and COX-2, encoded by different genes, are known to be present in the colon tumors of humans and rodents (17, 58, 59) and to catalyze the conversion of AA to PGs. Increased levels of COX-2 have been found in chemically induced colon tumors in F344 rats

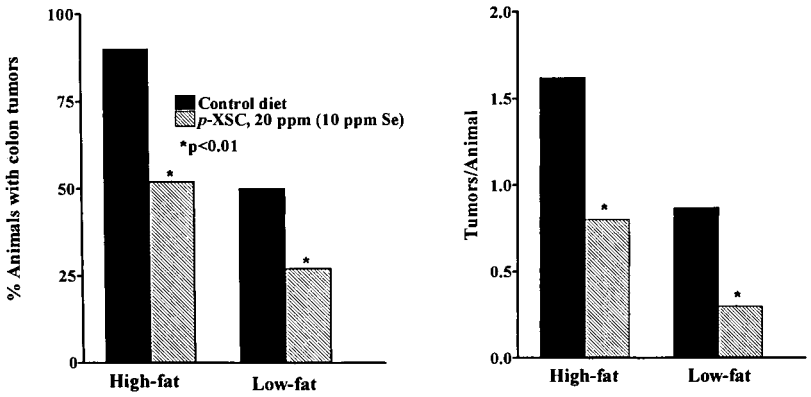


*Fig. 5.* Impact of high- and low-fat dietary regimen on *p*-XSC-induced colon tumor inhibition in F344 rats (72).

and intestinal adenomas from $Apc^{min}$ and $Apc^{\Delta 716}$ mice (56, 60, 61). Although both isozymes carry out essentially the same catalytic reaction, many of the inflammatory, inducible effects of COX appear to be mediated by COX-2, whereas the normal physiological functions of COX are mediated by COX-1 (60, 61). The expression of COX-1 does not fluctuate because of stimuli, whereas cytokines, mitogens, growth factors, and tumor promoters induce COX-2 expression. Prolonged administration of NSAIDs can cause unwanted side effects, such as gastrointestinal bleeding, ulceration, and renal toxicity, which are manifested mainly by the blocking of COX-1 activity.

Because NSAIDs can affect the activity of both COX-1 and COX-2, which accounts for their chemopreventive as well as their adverse side effects, one now needs to search for specific inhibitors of COX-2 that can serve as effective chemopreventive agents without causing side effects. In this context, it is noteworthy that the development of intestinal adenomas was strikingly (>6-fold) reduced in the COX-2 null mice compared with their occurrence in COX-2 wild-type mice; this suggests that COX-2 plays a key role in polyp formation (60, 61). Additional evidence in support of a role for COX-2 comes from studies showing that administration of the COX-2 inhibitor MF Tricyclic inhibited the incidence and size of intestinal tumors in $Apc^{\Delta 716}$ mice, a model in which a targeted truncation deletion in the tumor suppressor gene *APC* causes intestinal polyposis (62).

Studies from our laboratory indicate that celecoxib, a specific COX-2 inhibitor with significant anti-inflammatory and analgesic properties, significantly inhibited development of colonic preneoplastic lesions in rats (63). We had also observed that continuous administration of celecoxib throughout the initiation and postinitiation phases significantly suppressed the incidence and multiplicity of AOM-induced colonic adenocarcinomas in F344 rats (64). It is noteworthy that the degree of inhibition of colon carcinogenesis by celecoxib exceeded that seen with NSAIDs including aspirin, ibuprofen, sulindac, and piroxicam, which we tested previously for their chemopreventive potency in similar experimental design (Fig. 4). Thus, there are specific inhibitors of COX-2 that induce very few toxic effects but have increased chemopreventive potency. Although our understanding of the exact mechanism of the chemopreventive action of COX-2 inhibitors is still evolving, the development of preventive strategies on the basis of experimental studies will serve as a practical approach to the design of chemoprevention trials in humans. The results generated thus far make a strong case for the use of select COX-2 inhibitors as chemopreventive agents for the secondary prevention of colon cancer in high-risk individuals, such as patients with sporadic polyps and FAP.

## Organoselenium as a Chemopreventive Agent

Epidemiological studies have also pointed to an inverse association between dietary selenium intake and colon cancer risk in humans (65). Clark *et al.* (66) recently reported results from a randomized clinical trial in which they found that supplementation of selenium-enriched brewer's yeast reduced the incidence and mortality from cancer of the colon. This is corroborated by studies with selenium supplementation of the diet in chemically induced colon carcinogenesis in laboratory animals (67, 68). Humans ingest primarily organic forms of selenium, such as selenomethionine and selenocysteine by eating grains, vegetables, and animal products. Chemoprevention studies in laboratory animal models for mammary cancer have not revealed any significant differences between intake of inorganic and natural sources of selenium (68). Yet, chronic feeding of inorganic and certain organic forms of selenium at levels >5 ppm produced toxic effects. Therefore, substantial efforts were made to find and/or develop forms of organic selenium compounds that have maximal chemopreventive efficacy and lowest possible toxicity (68). Assays in our laboratory have indicated that certain synthetic organoselenium compounds hold great promise as chemopreventive agents, because they have been found to be superior to historically used selenium compounds, such as sodium selenite and selenomethionine. For example, in rats, BSC, but not its sulfur analogue, benzyl thiocyanate, inhibited AOM-induced colon carcinogenesis when administered during the initiation and/or postinitiation stages (69). To enhance the chemopreventive index of BSC, we conducted structure-activity assays and found that *p*-XSC was far less toxic, yet more effective, than BSC in inhibiting colon, mammary, and/or lung carcinogenesis (70–72). Because of the known tumor-promoting effects of high dietary fat intake, we also examined how fat intake would impact the chemopreventive efficacy of this organoselenium compound. This protocol is very important because secondary prevention of colon cancer by administration of chemopreventive agents alone may somewhat reduce the risk of colon cancer among high-risk individuals who consume a Western-style diet, but this protocol is likely most effective together with life-style changes, such as maintaining a low intake of dietary fat and preferably a higher intake of ω-3 fatty acids. Administration of *p*-XSC, along with a low-fat dietary regimen, inhibited the incidence and multiplicity of AOM-induced adenocarcinomas in F344 rats com-

pared with the findings in animals maintained on a high-fat diet plus *p*-XSC or a high-fat diet alone (Ref. 73; Fig. 5). This observation underscores the concept that a reduction in dietary fat may offer an important adjunct to chemopreventive efficacy in human colorectal cancer prevention trials involving not only organoselenium compounds but also other chemopreventive agents. These results make a strong case for the use of a low-fat dietary regimen, along with chemopreventive agents, as a desirable approach for primary prevention in the general population and for secondary prevention of colon cancer in high-risk individuals. This concept should be explored in a broad range of human trials involving various chemopreventive agents.

### Combinations of Low Doses of Various Chemopreventive Agents

There is increasing interest in the use of combinations of low doses of chemopreventive agents that differ in mode of action, rather than administering single agents as a means of obtaining increased efficacy and minimized toxicity. This approach is extremely important when a promising chemopreventive agent demonstrates significant efficacy but may produce toxic effects at higher doses. An example of combinations of agents producing positive results in laboratory animal models has been a study in which the NSAID piroxicam and DFMO, a specific irreversible enzyme-activated or suicide inhibitor of ODC, were evaluated for their chemopreventive efficacy. One of the mechanisms by which piroxicam inhibits colon carcinogenesis is through the suppression of PG synthesis that affects cell proliferation, tumor growth, and immune responsiveness. High intracellular polyamine levels and polyamine-synthetic enzyme activities have been shown to be high in neoplastic cells and in cells undergoing neoplastic transformation by carcinogens (74). The polycationic polyamines also stabilize DNA structures and affect DNA and protein synthesis. A critical step in polyamine biosynthesis is the ODC-catalyzed formation of putrescine from ornithine. ODC plays a role in normal and neoplastic cell proliferation in the intestinal mucosa and other organs (74). Several studies have demonstrated that tumor promoters induce ODC activity in the colonic mucosa, suggesting a relationship between tumor promotion and induction of ODC activity in the target organ (74, 75). Chemicals that inhibit induction of ODC or deactivate it are also chemopreventive. DFMO, a specific, mechanism-based irreversible inhibitor of ODC, has been shown to inhibit carcinogen-induced tumors in several target organs (74). Studies conducted in our laboratory demonstrated that administration of DFMO in the diet at concentrations of 2000 or 4000 ppm protects against AOM-induced colon carcinogenesis in a dose-dependent manner (76). In another study, we compared the chemopreventive efficacy of a combination of low doses of piroxicam (100 and 200 ppm) and low and high doses of DFMO (1000 and 2000 ppm, respectively) on AOM-induced colon carcinogenesis in F344 rats (Ref. 76; Fig. 6). The incidence and multiplicity of AOM-induced colon adenocarcinomas in F344 rats were significantly inhibited in rats given individually 200 and 400 ppm piroxicam and 2000 and 4000 ppm DFMO in the diet (76). Significantly, the administration of lowest doses, 100 ppm piroxicam plus 1000 ppm DFMO, dramatically inhibited colon tumorigenesis. An important finding of the study was that the lowest dose levels of piroxicam (100 ppm) and DFMO (1000 ppm), when administered together, were more effective in inhibiting the incidence and multiplicity of colon adenocarcinomas than administration of individual compounds, even at higher levels (Fig. 6). These data strongly support the view that the use of combinations of

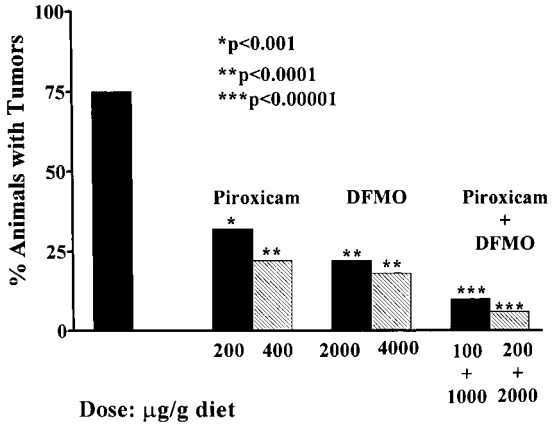


*Fig. 6.* Chemopreventive efficacy of piroxicam and DFMO administered together on AOM-induced colon carcinogenesis in F344 rats (76). Piroxicam was administered in the diet individually at 200 and 400 $\mu$g/g diet or DFMO individually at 2000 and 4000 $\mu$g/g diet. In the combination protocol, piroxicam at 100 $\mu$g/g diet plus DFMO at 1000 $\mu$g/g diet or piroxicam at 200 $\mu$g/g diet plus DFMO at 2000 $\mu$g/g diet were evaluated for their chemopreventive efficacy.

chemopreventive agents that have diverse actions should have beneficial applications in human cancer chemoprevention trials. This should be one of the approaches to future research and human intervention trials.

### Conclusions

An impressive body of observation supports the concept that dietary factors are key modulators of colon cancer. The roles of dietary fat as a risk factor and dietary fiber as a protective factor are highlighted as an example of the link between nutrition and cancer. Much progress in our understanding of the functional relationship between nutritional factors, including dietary fiber and fat as well as micronutrients and colon cancer, has been provided by epidemiological and diet intervention studies, relevant laboratory animal model studies, and investigations on mechanisms of tumor induction, progression, and inhibition. The emerging results reiterate our view that the primary prevention of colon cancer is to translate the findings from clinical epidemiological and laboratory studies into sound advice for patients and the public at large to reduce fat intake and increase fiber intake, especially cereals and grains.

Chemoprevention has the potential to be a major component of colon cancer control. Accumulating evidence indicates that NSAIDs including aspirin, ibuprofen, piroxicam and sulindac can reduce the incidence of colorectal cancer in laboratory animals and in humans. One of the mechanisms by which NSAIDs inhibit colon cancer is through the modulation of COX-1 and COX-2, which leads to a reduction of eicosanoid production, which in turn affects cell proliferation and tumor growth. These drugs can cause unwanted side effects, including gastrointestinal ulceration, bleeding, and renal toxicity, through the inhibition of constitutive COX-1 activity. Overexpression of COX-2 has been observed in colon tumors, and many commonly used NSAIDs have very little selectivity for COX-1 or COX-2; therefore, more specific yet minimally toxic inhibitors of COX-2 were developed and tested for chemopreventive efficacy. Celecoxib, a selective COX-2 inhibitor that induces very few toxic side effects, has been found to be significantly more effective than the commonly used NSAIDs in the chemoprevention of colon carcinogenesis in laboratory animal

models; it may thus be an effective chemopreventive agent against colon cancer.

Rapidly evolving progress in chemoprevention research in general has also brought about innovative approaches to the prevention of colon cancer. Studies in our laboratory have indicated that the synthetic organoselenium compound *p*-XSC holds great promise as a chemopreventive agent because it is far less toxic, yet more effective, than inorganic selenium in inhibiting colon carcinogenesis. More importantly, the chemopreventive effect of this agent is more pronounced when given along with a low-fat diet, thus making a strong case for the use of low-fat dietary regimens along with a chemopreventive agent as a desirable approach for primary prevention in the general population and for secondary prevention of colon cancer in high-risk individuals. Growing knowledge of the mechanisms by which chemopreventive agents act offers opportunities to use combinations of specific chemopreventive agents. A novel approach toward the chemoprevention of colon cancer is to coadminister two or more agents with different modes of action, the aggregate action of which would be significant while toxicity would be minimal. Efforts should be made to ensure a multidisciplinary approach to the trials planning of intervention trials in which advantage is taken of the information that was obtained from animal models mimicking development of cancer in humans. How to best use such knowledge in finding a specific prevention modality toward reducing cancer risk is a primary challenge for the future.

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