Chemoprophylaxis of Colon Cancer

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There is convincing evidence that chemoprevention has the potential to be a major component of colorectal cancer control. Experimental, epidemiologic, and clinical studies provide evidence that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the selective cyclooxygenase (COX)-2 inhibitors, including celecoxib and several phytochemicals, act as anticancer agents. However, several of these chemopreventive agents induce side effects at effective high dose levels. Low doses of atorvastatin and aspirin, or atorvastatin and celecoxib, or piroxicam and difluoromethylornithine administered in combination are more effective in inhibiting chemically induced colon adenocarcinomas in male F 344 rats than are high doses of these agents given individually.

Introduction

Colorectal cancer is one of the leading causes of cancer death in men and women in Western countries, including the United States, where approximately 145,290 new cases of colorectal cancer are estimated for the year 2005 [1]. Therefore, it is a major public health problem. A report by an expert panel assembled by the American Institute for Cancer Research and World Cancer Research Fund came to the scientific consensus that dietary factors, particularly caloric intake and saturated fat, may be of importance in the etiology of colon cancer [2]. Continuing population studies have revealed that diets particularly high in fish oil or fish reduce this risk [3]. In support of this observation, several animal studies using well-established colon cancer models provided experimental evidence that diets containing high levels of saturated fatty acids, such as those in Western diets, promote colon carcinogenesis, whereas diets high in ω-PUFAs (polyunsaturated fatty acids) had no such promoting effect [4–6]. Dietary epidemiologic studies have provided initial leads for the identification of naturally occurring candidate chemopreventive agents. The biologic rationale for an effect of these naturally occurring agents is strong because fruits, vegetables, and grains are the principal sources of micronutrients, including antioxidant nutrients and selenium, to cite a few, and other minor constituents such as organosulfur compounds, isoflavonoids, polyphenols, lignans, and phytates.

For secondary prevention in individuals who are at high risk for colon cancer and those who are diagnosed at early stages of colon cancer, and for subgroups with a particular genetic susceptibility to colon cancer, diet modification strategies alone are unlikely to produce rapid changes in cancer burden. An adjunctive approach to reduction in the incidence and/or further development of cancer progression is through chemoprevention, which refers to prevention of cancer by intervening with one or several chemical agents during initiation and progression stages in order to suppress or slow the carcinogenic process. This concept is different from that of chemotherapy because the latter involves the treatment of established cancer. The potential benefits of chemoprevention are substantial because the goals are to reduce cancer incidence and mortality, eliminate precancerous lesions, and increase the latency period.

This paper briefly summarizes our current knowledge of chemopreventive agents, both naturally occurring and synthetic analogues, evaluated against colon carcinogenesis in realistic preclinical models. Progress in the chemoprevention of colon cancer by these agents during the past two decades has been very impressive.

Nonsteroidal Anti-inflammatory Drugs

Epidemiologic and clinical studies

Epidemiologic studies have reported that individuals who regularly use aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have a lower incidence of adenomatous polyps and lower incidence of death from colorectal cancer compared with nonusers [7–9]. The first study that examined the relationship between aspirin use and colorectal cancer, conducted in Melbourne, Australia, demonstrated a highly significant protective effect in men and women [10]. Following this investigation, several other case–control studies likewise reported a protective effect of aspirin against colorectal cancer. The results of a large American Cancer Society Cancer Prevention Study of over 650,000 subjects are very consistent in showing a strong inverse relationship between use of aspirin and colon cancer risk [7,8]. The extent of protection appears to be dependent on both dose and duration of exposure. The risk of death from colorectal cancer of subjects who

reported taking aspirin at least 16 times per month was about 40% lower than that of those who reported not taking the drug.

Randomized clinical trials have established that sulindac [11] suppresses adenomatous polyps and causes regression of existing polyps in patients with familial adenomatous polyposis (FAP). Labayle *et al.* [12] reported that, in a randomized, placebo-controlled, double-blind crossover study in patients with FAP, administration of sulindac at a dosage of 300 mg/d for 6 to 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 [11]. Although the dosage of sulindac administered in these studies varied from 150 to 400 mg/d, most of the patients treated with this drug exhibited full remission, whereas some patients showed a partial response. By contrast, in some of the studies some FAP patients developed rectal carcinoma, despite ongoing therapy with sulindac [13,14], and adenomatous polyps resumed growth in FAP patients when NSAID treatment was stopped. With regard to sporadic adenomatous polyps NSAID prophylaxis produced no statistically significant difference in polyp size (regression of small <1 cm) among the 18 patients treated with sulindac (300 mg) for 4 months [15].

Preclinical studies

The development of strategies for prevention of colorectal cancer by chemopreventive agents has been markedly facilitated by the relevant laboratory animal models, including carcinogen-induced colon cancer mimicking the neoplastic process that occurs in humans. Azoxymethane (AOM), a potent inducer of carcinomas of the large intestine in various strains of rats, has been used extensively by many investigators to induce colon tumors and to study the effects of nutritional factors and chemopreventive agents in colon carcinogenesis [16].

Colons of Fischer (F 344) rats treated with AOM seem to have light and electron microscopic morphology as well as histochemical properties that are quite similar to those of humans, and the biologic behavior of AOM-induced rat colon carcinomas is similar to that of human colon carcinomas [16]. Other characteristics of the human disease process reflected in the AOM rat model are the occurrence of adenomas and adenocarcinomas and aberrant crypt foci (ACF), which are recognized as early appearing preneoplastic lesions [16].

Ample and consistent experimental evidence from animal model studies indicates that NSAIDs, including indomethacin, piroxicam, sulindac, aspirin, ibuprofen, and ketoprofen, inhibit chemically induced colon cancer. Pioneering studies by Narisawa *et al.* [17,18] and Pollard and Luckert [19] demonstrate that indomethacin and piroxicam, administered to rodents in drinking water, diet, or intraperitoneally, inhibited colon tumors induced by a variety of carcinogens. Since that time, a number of investigators have evaluated the preventive efficacy of several NSAIDs against colon carcinogenesis [20–35]. These studies have demonstrated that administration of NSAIDs, including aspirin, ibuprofen, piroxicam, ketoprofen, and sulindac, during the initiation and post-initiation stages of carcinogenesis suppressed the incidence and multiplicity of colon tumors. Of particular interest is that aspirin, piroxicam, and sulindac even reduced spontaneous intestinal tumorigenesis in APC Min mice with germline mutation in the *APC* gene, which is genetically predisposed to develop intestinal tumors [31–34]. In conclusion, irrespective of the type of NSAID tested (indomethacin, aspirin, ibuprofen, piroxicam, ketoprofen, and sulindac) and variations in the timing of treatment (initiation/post-initiation, or promotion and progression phase) these agents suppressed the incidence and multiplicity of colon tumors (Fig. 1) [35]. The studies in rodents proved conclusively that aspirin and other conventional NSAIDs inhibit chemically induced carcinogenesis in rats and mice. In our laboratory experiments, nonselective NSAIDs reduced colon tumorigenesis by 40% to 70% in a dose-dependent manner, when tested at greater than 80% of the tolerable dose levels of aspirin, ibuprofen, ketoprofen, piroxicam, and sulindac [35]. Because commonly used NSAIDs can inhibit cyclooxygenase (COX)-1 and COX-2, which accounts for their chemopreventive application and their adverse side effects, several COX-2 inhibitors have been identified and tested for their efficacy [25–30]. Our studies with celecoxib in the rat model indicate that high doses of this COX-2 inhibitor (1500 ppm in food) reduced tumors by 90% and were better tolerated (~35% of tolerable dose) than comparable (80% tolerable) doses of nonselective NSAIDs [25,28]. Selective COX-2 inhibitors, such as celecoxib, inhibit tumor development in APC Min mice [36••]. These models mimic the rapid development of adenomatous polyps that affect humans with germline inactivation of one *APC* gene but differ from FAP in that the mouse tumors occur predominantly (>95%) in the small intestine and rarely in the colon.

Enhancing the efficacy of NSAIDs

Several approaches have been developed to enhance the selectivity and reduce the toxicity of NSAIDs, and specifically COX-2 inhibitors. One of the approaches, which is a drug improvement strategy, is to develop novel agents that spare COX-1 functions or compensate for COX-1 activities but suppress inflammation in chronic arthritis patients while thus avoiding the most serious gastrointestinal toxic effects. A valid approach is to identify and develop novel NSAIDs, such as nitric oxide (NO)-NSAIDs, phytochemicals with anti-inflammatory activities, and low-dose NSAIDs, and combine them with other chemopreventive/dietary measures for the prevention of cancer.

NO-NSAIDs consist of a known NSAID molecule and an NO-releasing group (typically-NO_2) linked to it via a chemical spacer. One of the important rationales for their

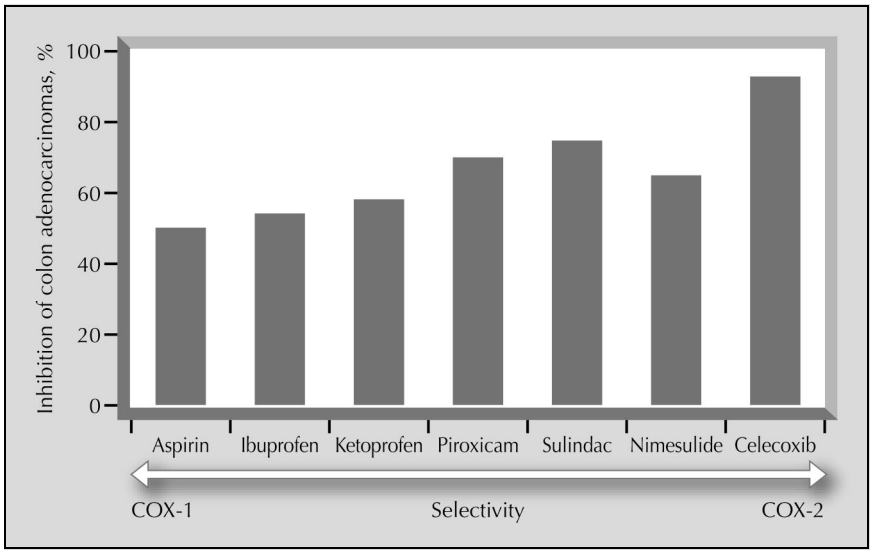

Figure 1. Chemopreventive efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase (COX)-2 selective inhibitors on azoxymethane (AOM)-induced colon adenocarcinoma formation. Aspirin, ibuprofen, ketoprofen, piroxicam, and sulindac were given at 80% tolerable dose, and nimesulide and celecoxib were given at approximately 35% to 40% tolerable dose during initiation and post-initiation stages of colon carcinogenesis.

development was the observation that NO possesses several properties similar to those of prostaglandins (PGs) derived from COX-1 activities in gastric mucosa. Like PGs, NO increases mucosal blood flow, mucus release, and repair of the mucosa, whereas it inhibits neutrophil activation and adherence. Thus, NO can compensate for COX-1/PG inhibition by conventional NSAIDs. Coupling an NO-releasing moiety to an NSAID might deliver NO to the site of NSAID-induced damage and thus protect against gastric toxicity.

With regard to cancer, NO-NSAIDs have been observed to inhibit tumor cell growth by stimulating apoptosis and blocking cell proliferation from G0-G1 to S cell cycle transition [37]. In vitro studies with human colon cancer cell lines suggest that NO-aspirin, NO-ibuprofen, and NO-sulindac are potent inducers of apoptosis. Importantly, these NO-NSAIDs are several folds more potent than the conventional NSAIDs [37]. However, the efficacy of NO-NSAIDs against the various types of cancers has not been fully established in preclinical models. Recently, we tested a number of NO-NSAIDs for their tolerability and efficacy toward chemically induced preneoplastic lesions of the colon [38]. In F 344 rats, chronic feeding of NO-aspirin, NO-flurobiprofen, NO-sulindac, and NO-ibuprofen is well tolerated even at very high dose levels compared with those of their parent compounds. Importantly, these NO-NSAIDs suppressed AOM-induced colonic ACF formation in the rat [38]. A number of studies are evaluating the chemopreventive potential of NO-NSAIDs against various types of cancer.

Phytochemicals with anti-inflammatory activities

The observation that dietary components exhibit biochemical and physiologic properties analogous to those of NSAIDs has fostered increased interest in research on the use of such dietary substances as potential agents in reducing the risk of cancer. Agents such as curcumin, phenylethyl methylcaffeate, ursolic acid, and oleanolic acid have been shown to possess anti-inflammatory activity [39]. Importantly, most of these phytochemicals induce anti-inflammatory activities by modulating COX activities similar to those induced by synthetic NSAIDs, but they are less toxic than the NSAIDs. Among naturally occurring anti-inflammatory agents, curcumin was extensively studied and proved to be an inhibitor of several types of chemically induced neoplasia [40–42]. Dietary administration of curcumin reduces formation of focal areas of dysplasia and aberrant crypt foci in the colon, which are early preneoplastic lesions in rodents [42]. Continuous feeding of 0.2% curcumin during the initiation and post-initiation stages of AOM-induced colon carcinogenesis reduced adenocarcinoma incidence, multiplicity, and the total tumor burden in male F 344 rats [40]. Curcumin, given as a dietary supplement during the promotion/progression period, dramatically inhibited colon tumorigenesis in a dose-dependent manner [42].

Phenylethyl caffeate and its analogue, phenylethyl methylcaffeate, are major components of propolis in the honey beehive that possess anti-inflammatory activities and inhibit AOM-induced colonic ACF, adenocarcinoma, skin carcinogenesis, and also intestinal polyp formation in APC Min mice [43–46]. Triterpenoids, such as oleanolic acid and its analogues, have suppressed COX-2 expression and activity in RAW 264.7 cells and inhibit AOM-induced colonic ACF formation in a dose-dependent manner in rats [39]. Importantly, curcumin, phenylethyl methylcaffeate, ursolic acid, oleanolic acid, and their analogues have no known side effects like those seen with synthetic, conventional NSAIDs. The inhibitory effect of curcumin and other anti-inflammatory phytochemicals is in part associated with increased apoptosis, suggesting that increased cell death may be one of the mechanisms by which these agents block carcinogenesis. This information suggests that phytochemicals that possess anti-inflammatory activity may retard growth and/or development of existing neoplastic lesions in the colon, and these agents may be effective chemopreventive agents

for individuals at high risk for colon cancer development, such as patients with polyps.

With regard to their mode of action, curcumin, and phenylethyl caffeate exhibit an array of metabolic, cellular, and molecular activities, including inhibition of arachidonic acid (AA) formation and its further metabolism to eicosanoids. In our assays, dietary administration of these agents significantly inhibited PLA_2 and PI-PLC in the colonic mucosa and tumor tissues, leading to the release of AA from phospholipids; they also altered COX activity and modified PGE_2 levels [47–49]. In contrast to NSAIDs, dietary curcumin or phenylethyl methylcaffeate inhibit lipoxygenase (LOX) activity, and block the production of the LOX metabolites, 5(*S*)-, 8(*S*)-, 12(*S*)- and 15(*S*)-hydroxyeicosatetraenoic acids (HETEs), in the colonic mucosa and in tumors [40–42]. Importantly, 12(*S*)-HETE is known to promote tumor cell adhesion and to stimulate the spreading of tumor cells, thus augmenting metastatic potential [47]. It is important to note that phenylethyl methylcaffeate preferentially suppresses the 12(*S*)-HETE formation in AOM-induced colonic tumors [43]. Other studies indicate that curcumin and phenylethyl methylcaffeate also inhibit several mediators and enzymes involved in the mitogenic signal transduction pathways of the cell and in AP-1 and NFκB activation [48,49]. Overall, naturally occurring anti-inflammatory agents predominantly block the expression of COX-2 activity by acting on upstream signaling pathways at the level of mRNA, suggesting that the mode of action of these agents is somewhat different from that of the NSAIDs, which modulate the COX-2 protein. This difference in mode of action between these anti-inflammatory phytochemicals and NSAIDs may, in part, explain the lack of toxicity of these agents in comparison with NSAIDs.

Organosulfur Compounds

It is apparent that foodstuffs are a source of naturally occurring chemopreventive agents that hinder the formation of carcinogens from precursors in the body or that act protectively to lessen or eliminate the effects of carcinogens and tumor promoters. Green and yellow vegetables, including cabbage, Brussels sprouts, and other cruciferous vegetables, contain several organosulfur compounds, including isothiocyanates and dithiolethiones. Additional work has also focused on organosulfur compounds found in garlic (*Allium* species), that is, diallyl sulfide, diallyl disulfide, and diallyl trisulfide. Several of these agents have been tested in short-term screening and in long-term efficacy studies in laboratory animals for their chemopreventive properties. Among dithiolethiones, oltipraz, a substituted dithiolethione (5-2(pyrazinyl)-4-methyl-1,2-dithiole-3-thione), and anethole trithione have been evaluated for their potential chemopreventive properties in a colon cancer model [50]. In addition, diallyl disulfide present in garlic and onions was tested for its efficacy. The dietary administration of oltipraz, anethole trithione, and diallyl sulfide significantly suppressed chemically induced colon tumors in laboratory animal models in a dose-dependent manner [50]. With regard to modes of action of these agents, the inhibition of colon carcinogenesis was associated with an induction of colonic mucosal glutathione-*S*-transferase and NADP(H)-dependent quinine reductase activities that are involved in carcinogen detoxification and elimination.

Combination of Low Doses of NSAIDs with other Chemopreventive Agents

An important strategy to improve the balance of benefits and risks associated with NSAID use is to identify combinations of agents with different modes of action that are effective at very low doses. 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 was a study in which piroxicam, an NSAID, and difluoromethylornithine (DFMO), a specific, irreversible enzyme-activated or suicide inhibitor of ornithine decarboxylase (ODC), were evaluated for their chemopreventive efficacy [51•]. We used combined administration of low and high doses of piroxicam (100 and 200 ppm) and low and high doses of DFMO (1000 and 2000 ppm, respectively) in AOM-induced colon carcinogenesis in F 344 rats [51•]. Incidence and multiplicity of AOM-induced colon adenocarcinomas in F 344 rats were significantly inhibited in F 344 rats given 200 or 400 ppm of piroxicam and 2000 or 4000 ppm of DFMO in their diet. An important finding of the study was that the low dose levels of piroxicam (100 ppm) and DFMO (1000 ppm) administered together were more effective in inhibiting the incidence and multiplicity of colon adenocarcinomas than administration of the individual compounds even at higher levels (Fig. 2).

In other studies we have shown that combination of much lower doses of sulindac, an NSAID, and the cholesterol-lowering drug lovastatin suppressed chemically induced colon cancer in rodents and stimulated apoptosis in human tumor cells when the drugs were given in combination than when either drug was given alone [52,53•]. In addition, aspirin in combination with DFMO has been tested against colon carcinogenesis [54]. Our recent study provided convincing evidence that a very low dose of lovastatin in combination with a low dose of celecoxib suppressed invasive and noninvasive adenocarcinomas of the colon in a chemically induced colon cancer model [55••]. These data strongly support the view that combinations of chemopreventive agents that have diverse mechanisms of action can have beneficial applications in human cancer chemoprevention trials. This should be one of the approaches to future research and human intervention trials.

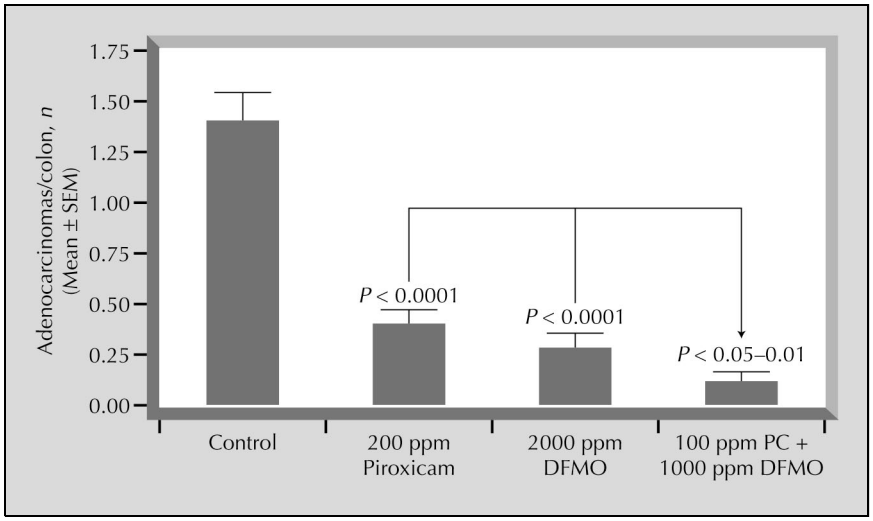

Figure 2. Chemopreventive effect of piroxicam (NSAID) and difluoromethylornithine (DFMO, an ornithine decarboxylase inhibitor) individually and in combination on azoxymethane (AOM)-induced colon adenocarcinoma formation in F 344 rats.

Combination of Low Doses of Celecoxib and Omega-3 Fatty Acid–Rich Diet

Based on the efficacy of NSAIDs and COX-2 inhibitors in preclinical applications, we believe that intervention with chemopreventive agents alone may not be sufficient for secondary prevention of cancer in high-risk patients. It is well established that Western-style diets with high animal fat content constitute a significant risk factor for many cancers, including cancer of the colon [4–6]. For example, in preclinical models, we have shown that Western-style diets containing 20% mixed lipids pose a four- to fivefold greater risk for colon cancer compared with a low-fat diet (5% corn oil) traditionally used in chemoprevention studies, or a diet rich in ω-3 fatty acids (20%) [4–6]. Future trials involving administration of NSAIDs at low doses along with low-risk diets would provide an ideal strategy for the prevention of colorectal cancer. In support of this concept, our recent study clearly demonstrates that a high-fat diet containing ω-3 PUFAs (high-fat fish oil [HFFO]) induced fewer colon tumors than did a high-fat diet containing mixed lipids ([HFML] saturated and unsaturated fats). A very low dose of celecoxib administered in the HFFO diet caused a significant inhibition of colon adenocarcinomas as compared with that of a very low dose of celecoxib in the HFML diet [56••]. These studies strongly support the view that low doses of celecoxib in a ω-3 PUFA–rich diet are a highly promising approach for human clinical trials.

Conclusions

An impressive body of evidence supports the concept that chemoprophylaxis, also called chemoprevention, has the potential to be a major component of colon cancer control. Accumulating evidence indicates that conventional NSAIDs, including aspirin, sulindac, piroxicam, ibuprofen, and phytochemicals with anti-inflammatory properties, including curcumin, caffeic acid esters, isothiocyanates and garlic compounds, have the potential to act as chemopreventive agents 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 that, in turn, affects cell proliferation and tumor growth. These drugs, however, can cause unwanted adverse 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, but most commonly used NSAIDs have very little selectivity between COX-1 and 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, has been found to be significantly more effective than the commonly used NSAIDs in the chemoprevention of experimentally induced colon carcinogenesis.

Rapidly evolving progress in chemoprevention research in general has brought about innovative approaches toward the prevention of colon cancer. Understanding the mechanisms of action of NSAIDs and other chemopreventive agents, including statins, offers opportunities to use a combination of specific agents. Developing agents, such as selective COX-2 inhibitors, NO-NSAIDs, and phytochemicals with anti-inflammatory properties, and combinations of low doses of NSAIDs and statins will be a practical approach for colon cancer prevention. Efforts should be made to ensure a multidisciplinary approach to the planning of interventional trials in which advantage is taken of the information obtained from animal models mimicking development of cancer in humans. How best to use such knowledge in finding a specific prevention modality toward reducing cancer risk is the primary challenge for the future.

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This study concluded that administration of high doses of atorvastatin, celecoxib, and aspirin individually and low doses of these agents in combination in the diet significantly inhibited chemically induced colon adenocarcinomas in a realistic rat model. Low doses of atorvastatin and celecoxib and atorvastatin and aspirin in combination are more effective than the high doses of these agents given individually.

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The results strongly indicate that very low, low, and moderately low levels of celecoxib administered in diets containing saturated fats or omega-3 fatty acids (ω-3 PUFAs) suppressed colon carcinogenesis. More importantly, the inhibition of colon adenocarcinomas was more pronounced in animals given very low doses of celecoxib in ω-3 PUFAs as compared with the effects of very low dose celecoxib given in a saturated fat diet.