

Chemoprophylaxis in gastrointestinal tumors

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Abstract. – Chemoprevention of gastrointestinal tumors uses natural or synthetic agents to arrest, retard or reverse the carcinogenesis process. The prospect of prevention is clearly appealing, especially for colorectal cancer (CRC), that represents the second most common cause of cancer-related death in the Western world. Aspirin is the best studied chemopreventive agent for CRC, with randomized trials demonstrating its efficacy in reducing recurrence of colorectal adenomas in higher risk patients. Optimal chemoprevention requires long-term use and high dose of aspirin that may increase the risk of gastrointestinal bleeding. Other nonsteroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors also reduce the incidence of colonic adenomas, but they are associated with gastrointestinal harms and important cardiovascular events, respectively. Furthermore, cumulative epidemiological and observational data suggest the potential role of hormones as a chemoprotective agent for CRC. The usefulness of folic acid, calcium, and vitamin D awaits further evaluation. Interestingly, combining different agents may maximize effectiveness while limiting drug toxicity. Although many agents have shown positive results in the field of chemoprevention, it cannot yet be accepted as standard medical practice for CRC.

In the present review we discuss the most promising agents in CRC chemoprevention, together with their potential mechanisms of action in tumor inhibition.

Key Words:

Chemoprevention, Colorectal cancer, NSAIDs, Agent combinations.

Introduction

Gastrointestinal tumors, including esophageal, gastric and bowel cancers, together make up 18 per cent of new cancer diagnosis. The prospect of prevention is clearly appealing, especially for colorectal cancer (CRC) because it is such a widely

prevalent disease associated with considerable morbidity and mortality. In fact, every year, about 800,000 people worldwide are diagnosed with CRC, and nearly 450,000 died due to this disease, representing the second most common cause of cancer-related death in the Western world¹. CRC screening is the cornerstone of prevention. The removal of premalignant adenomas by colonoscopic polypectomy is an effective prevention method, and reduces cancer mortality in susceptible patients by 30-40%²; however, it is not yet available widely even in developed countries.

Cancer represents a late, non-obligate stage of carcinogenesis, a chronic process that provides time and targets for chemoprevention. Cancer chemoprevention refers to the use of natural or synthetic agents to arrest, retard or reverse the carcinogenesis process before the onset of the clinical disease³. In this way, chemoprevention is medicine, and it may be considered as the chemotherapy of dysplasia or intraepithelial neoplasia. CRC has a natural history of evolution from normal mucosa to adenoma to overt cancer that spans on average 10-20 years, thereby providing a window of opportunity for effective intervention and prevention.

The rationale for developing chemopreventive approaches to prevent CRC came from epidemiologic and observational studies indicating that long term ingestion of aspirin could reduce CRC mortality⁴. More than 200 agents have been considered, but, currently, only celecoxib is FDA approved for chemoprevention of CRC and only for high-risk patients with Familial Adenomatous Polyposis (FAP). The ideal chemopreventive agent should fulfill the following criteria: (1) the drug must be effective; (2) it should have a convenient dosing schedule; (3) it should have an acceptable safety profile; (4) it should be easy administered; and (5) it should be inexpensive.

In the present review we discuss the most promising agents in CRC chemoprevention, together with the potential mechanisms underlying their anti-neoplastic effects.

Aspirin and Nonsteroidal Anti-Inflammatory Drugs in CRC Chemoprevention

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclo-oxygenase (COX) enzymes, COX-1 and COX-2, which catalyse the conversion of arachidonic acid to prostaglandins. COX-1 is constitutively expressed in many tissues, whereas COX-2 is induced by cytokines, growth factors, and mitogens. Tumor inhibition by NSAIDs may be mediated by distinct cellular processes. These processes involve the ability of NSAIDs to restore apoptosis, induce cell-cycle arrest, and inhibit angiogenesis⁵. Because COX-2 expression is increased in up to 90% of sporadic colon carcinoma and 40% of colonic adenomas, but not in normal colonic mucosa⁶, NSAIDs were presumed to mediate apoptosis via COX-2 inhibition. A mechanism for COX-2-dependent NSAID-induced apoptosis is provided by arachidonic acid (Figure 1). Treatment of colorectal

cancer cells with various NSAIDs results in inhibition of COX-2 and a dramatic increase in the concentration of arachidonic acid, the main substrate for COX-2⁷. This build-up of arachidonic acid stimulates the enzyme sphingomyelinase to convert sphingomyelin to ceramide, a potent inducer of apoptosis⁸. Moreover, arachidonic acid can alter mitochondrial permeability and cause cytochrome C release, leading to apoptosis⁹. The relevance of COX-2 for adenoma formation was genetically demonstrated by a reduced number of adenomas in APC^{min/+} mice, the mouse model for FAP, with an additional targeted deletion of COX-2¹⁰. However, compounds that do not inhibit COX-2, such as sulindac sulphone, also induce apoptosis *in vitro* and inhibit colorectal carcinogenesis in animal model¹¹. This suggests that other targets of NSAIDs that are common to some neoplastic cells may play a part in NSAID-mediated apoptosis (Figure 1). NSAIDs could promote apoptosis by inhibiting the activation of transcription factor NFκB, which promotes cell

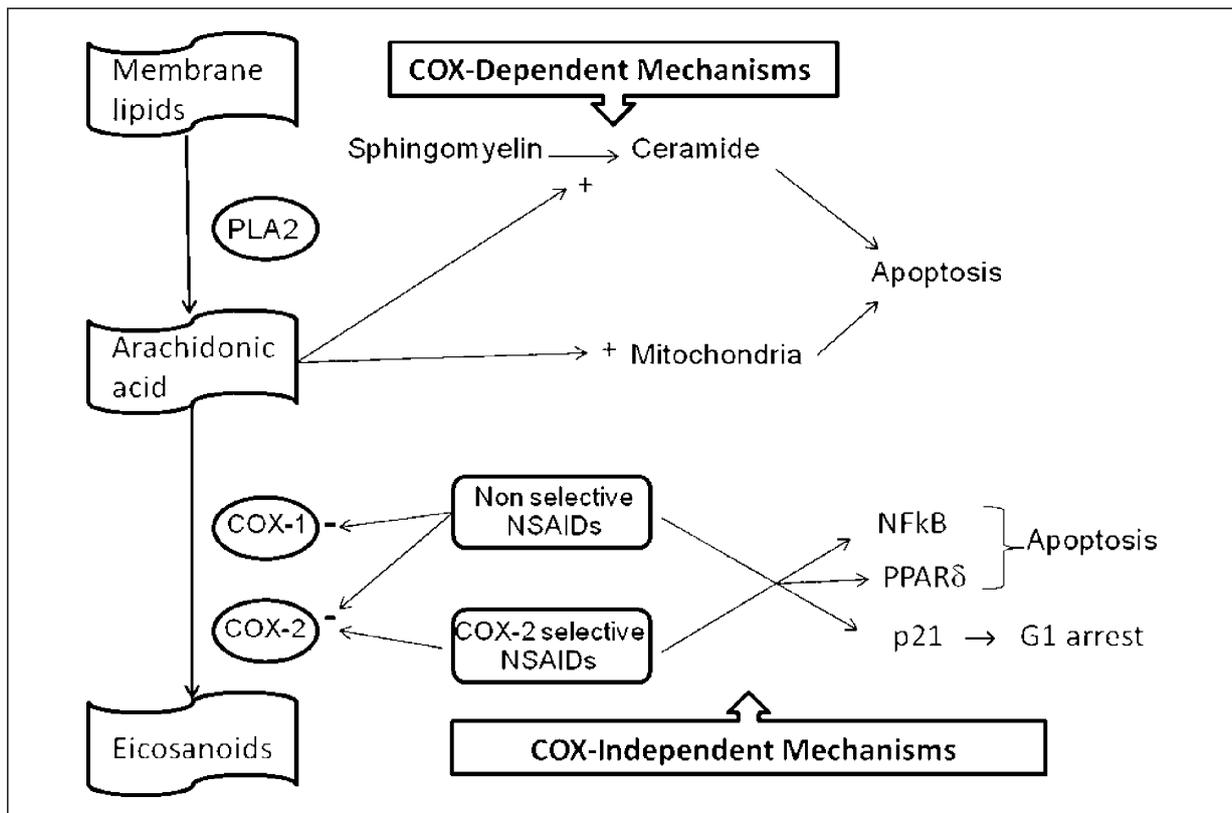


Figure 1. Mechanisms underlying the anti-neoplastic effects of NSAIDs. NSAIDs, inhibiting COX-enzymes, determine a dramatic increase in concentration of arachidonic acid, which stimulates the synthesis of ceramide and causes cytochrome C release, leading to apoptosis. NSAIDs can promote apoptosis also in a COX-independent manner, inhibiting NFκB and PPARδ directly. Finally, NSAIDs induce p21 expression resulting in G1 cell-cycle arrest.

survival and proliferation¹². Another potential COX-independent mechanism of NSAID-mediated apoptosis involves the peroxisome-proliferator-activated receptor δ (PPAR δ). NSAIDs may interfere with the binding of PPAR δ to DNA, so that the cell is left unable to transcribe the genes necessary for its survival¹³. Moreover, it has been shown that sulindac induce expression of p21 in colon cancer cells, resulting in G1 arrest¹⁴. This mechanism may account for chemopreventive effects of sulindac in addition to its effect on apoptosis.

Supportive evidence for the role of aspirin and other NSAIDs in the prevention of CRC has been derived from more than 200 randomized, placebo-controlled animal studies, in which the administration of various NSAIDs resulted in fewer tumors per animal and fewer animals with tumors¹⁵. Epidemiologic observations and population-based studies also showed that long-term use of aspirin and other NSAIDs reduced the risk of CRC¹⁶⁻¹⁹. The first population-based case-control study showed a relative risk (RR) reduction of 0.53% for CRC among regular aspirin users compared with non-aspirin consumers¹⁶. The long duration of use required to prevent invasive cancer may reflect the time required for cancer to develop from precursor lesions.

Colorectal adenomas, the precursors to most colorectal cancers, would be expected to reflect the chemopreventive effects of aspirin sooner than the invasive cancers because these lesions occur much earlier in the carcinogenic pathway.

Four randomized controlled trials in patients with previous adenoma or CRC have shown significant efficacy in preventing polyp recurrence at daily dosages of 81-325 mg/day of aspirin. Sandler et al randomized more than 600 patients with a recent history of CRC to receive aspirin (325 mg/day) or placebo. A statistically significant reduction in the incidence of colorectal adenomas was found in the treatment arm (17% vs 27%)²⁰. Baron et al. also found a reduction in the risk of recurrent adenomas in one of the aspirin treatment arms compared with placebo. Surprisingly, only the lower dose of aspirin (81 mg daily) had a statistically significant effect. The higher dose of aspirin (325 mg daily) reduced the risk of 4% but it was not significant²¹. Notably, protection against advanced neoplasms (adenomas measuring at least 1 cm in diameter or with tubulovillous or villous features, severe dysplasia, or invasive cancer) was more pronounced than the effect on risk of recurrence of any ade-

noma (41% and 17%, respectively)²¹. Ben-amouzig et al. randomly assigned 272 patients with a history of colorectal adenoma to daily lysine acetylsalicylate (160 or 300 mg/day) or placebo for 4 years²². Both dosages were effective in reducing polyp recurrence at 1 year in 27% (95% CI, 0.52-1.04). In the UK-CAP trial, 945 patients with an adenoma removed in the 6 months before recruitment were randomized to 1 of 4 treatment arms using a 2 \times 2 factorial design (aspirin 300 mg/day; folate supplements 0.5 mg/day; aspirin plus folate; placebo)²³. Patients receiving aspirin had a 21% reduction in colorectal adenoma recurrence compared to placebo. Folic acid supplementation had no effect on development of recurrent colorectal adenoma²³.

To better characterize the apparent chemopreventive effect of aspirin in the large bowel, Cole et al. performed a meta-analysis of all available randomized clinical trials that investigated whether aspirin reduces the risk of colorectal adenomas²⁴. Authors identified four clinical trials with nearly 3,000 randomly assigned participants. Each trial evaluated aspirin for secondary prevention of colorectal adenomas, at doses ranged from 81 to 325 mg/d. They found a statistically significant 17% decrease in the relative risk of adenoma for aspirin in any dose vs placebo, which correspond to a 6.7% absolute risk reduction²⁴. They also observed a 28% decrease in the relative risk of advanced lesions (ie, tubulovillous adenomas, villous adenomas, adenomas \geq 1 cm in diameter, adenomas with high-grade dysplasia, or invasive cancer). Only two studies investigated lower-dose aspirin, and, therefore, dose-response patterns were not interpretable. In addition, analysis of cardiovascular and bleeding events was limited by the small numbers of events observed in any one trial, and one trial was excluded from the analysis of adverse events²⁴.

However, it has been estimated that about 16,500 NSAID-related deaths occur every year in the United States, due to gastrointestinal and renal toxicity of these drugs. Thereby, agents with more acceptable side effect profiles are required for CRC chemoprevention.

COX-2 Inhibitors in CRC Chemoprevention

COX-2 is an inducible enzyme, that has been shown to be overexpressed in CRC and adenomas in humans⁶, and was demonstrated in knockout mice to be a controlling factor in the formation of adenomas¹⁰. Concerns regarding the gas-

trointestinal and other toxicities of aspirin drove the quest for selective COX-2 inhibitors (coxibs) that would have the antineoplastic actions of aspirin without its toxicity.

In patients with FAP, the number of colorectal polyps has been shown to be reduced by 28% in patients treated with 400 mg of celecoxib twice daily for 6 months as compared with a reduction of 4.5% in the placebo group ($p=0.003$)²⁵. Celecoxib is now licensed in the USA for the reduction of polyp numbers in FAP, in conjunction with endoscopic surveillance or surgery. Three prospective, randomized, placebo-controlled trials were designed to determine whether coxibs also prevent sporadic colorectal adenomas.

The Adenomatous Polyp Prevention on Vioxx (APPROVe) trial recruited more than 2500 patients with a history of colorectal adenomas²⁶. They received 25 mg of rofecoxib daily or placebo for 3 years. A 24% reduction in adenoma recurrence was found in the treatment group²⁶. The Adenoma Prevention with Celecoxib (APC) trial randomized more than 2000 patients with prior history of adenoma to receive placebo or celecoxib (200 or 400 mg twice daily). At 3 years of follow-up, Authors observed a significant reduction in the incidence of new adenomas in the treatment arms²⁷. In the Prevention of Sporadic Adenomatous Polyps (PreSAP), Authors randomly (3:2) assigned 1561 subjects who had had adenomas removed before enrollment to receive celecoxib (400 mg) or placebo daily²⁸. The cumulative rate of adenomas detected through year 3 was 33.6% in the celecoxib group and 49.3% in the placebo group (<0.001)²⁸. Although all three trials showed that coxibs reduced polyp recurrence, in the APPROVe and the APC studies this efficacy was associated with an increased risk of cardiovascular events (such as myocardial infarction, stroke, and heart failure). Because of the severity of the drug-related cardiovascular events, celecoxib cannot be recommended for routine prevention of sporadic colorectal adenomas or CRC, while rofecoxib was withdrawn from the market. The mechanism responsible for cardiovascular toxicity of this class of drugs is related in part to the evidence that selective inhibition of COX-2 can block the production of prostacyclin without affecting the synthesis of thromboxane A_2 ²⁹, thereby potentially creating a prothrombotic state. On the contrary, in the PreSAP trial, which involved a daily dose of 400 mg of celecoxib, no apparent increase in cardiovascular risk has been shown^{28,30}. This suggests that

once-daily dosing at 400 mg is safer than a twice-daily dose of 200 mg, even if this assertion cannot be conclusive because neither of these trials was designed or powered to assess cardiovascular toxicity.

Determining specific subpopulations who may benefit from these drugs, as compared with populations who are at increased risk for side effects, remains an important objective.

Calcium and Vitamin D

Experimental and epidemiological evidence suggests that calcium may prevent colorectal adenomas, even if the results regarding the relationship between calcium intake and CRC risk have not always been consistent. The mechanisms responsible for the protective effect of calcium are based on its bile acid-binding capability and direct action on intracellular events (cell proliferation, differentiation, death).

A systematic review of the literature was conducted to assess the effect of supplementary dietary calcium on the development of CRC and adenomatous polyps in healthy adults and adults at higher risk of colon cancer (family history, previous adenomatous polyps, or inflammatory bowel disease). Two well conducted randomised placebo-controlled intervention studies involving 1346 subjects followed for 3-4 years were identified. The doses of supplementary elemental calcium used were 1200 mg daily for a mean duration of 4 years, and 2000 mg/day for three years. A reduction on the development of colorectal adenomatous polyps for dietary supplementation of at least 1200 mg elemental calcium per day was found (OR 0.74, CI 0.58,0.95) when the results from both trials were combined³¹. Nevertheless, Authors concluded that this does not constitute sufficient evidence to recommend the general use of calcium supplements to prevent colorectal cancer³¹.

Recently, also vitamin D has received attention as a possible cancer-preventive agent. Vitamin D suppresses proliferation and promotes differentiation and apoptosis in colorectal cells, through effects on growth factors, cell cycle regulation and apoptosis. A daily intake of 1000 IU of vitamin D was associated with 50% lower risk of CRC³². The multicenter Women's Health Initiative study involved 36,282 postmenopausal women receiving calcium (500 mg bid) plus vitamin D supplementation (200 IU bid) with a mean follow-up of 7 years; CRC was a secondary endpoint³³. The study reported no association be-

tween supplementation and CRC. The relatively short duration of follow-up and suboptimal doses of calcium and vitamin D were suggested to account for the negative results of this trial. For primary cancer prevention, Gorham et al. propose intakes of vitamin D of 1,000 to 2,000 IU/d³⁴. However, dose-limiting hypercalcemic effects have proved a major obstacle to the development of natural vitamin D as a cancer chemopreventive agent. The National Cancer Institute is no longer exploring it in chemoprevention clinical trials.

Folic Acid

Considerable epidemiological evidence suggests that a low-folate diet is associated with an increased risk of colorectal neoplasia, particularly in concert with alcohol, which can antagonize the metabolism of folate³⁵. A low serum folate level, in the form of 5-methyl-tetrahydrofolate, may limit the supply of methyl groups required for DNA methylation. DNA hypomethylation is an early event in colon carcinogenesis. However, in a double-blind, randomized, placebo-controlled, phase III clinical trial, folic acid supplementation (1 mg daily for up to 6 years) failed to reduce colorectal adenoma recurrence and may have increased the risk of advanced lesions among individuals with previously removed adenomas³⁶. Contrary, a randomized, single institution, double-blind placebo controlled trial demonstrated that high dose folic acid supplementation (5 mg/d for 3 years) is associated with a significant reduction in the recurrence of colonic adenomas³⁷. Although the reasons for these controversial issues are not fully understood, one possibility could be attributed to the dual modulatory effect of folic acid on carcinogenesis: it may promote progression of established neoplasms, while it could protect against development of premalignant lesion³⁸.

Hormone Replacement Therapy

Several observational studies reported a protective effect of hormone replacement therapy (HRT) on colorectal cancer risk³⁹. The findings from the randomized Women's Health Initiative (WHI) trial, including healthy women who received oral combined estrogen-progestin regimen, confirmed the reduced incidence of colorectal cancer (hazard ratio, 0.56; 95% CI, 0.38-0.81; $P=0.003$)⁴⁰. However, in the hormone group the invasive CRCs were more advanced and with a greater number of positive lymph nodes.

Estrogen may prevent CRC by: decreasing the production of secondary bile acids, decreasing the production of IGF-1, and exerting a direct effect on the epithelium.

Since the magnitude of benefit from HRT is offset by its numerous risks (mainly, cardiovascular events and breast cancer), taking HRT to prevent colorectal cancer is not usually recommended.

Combination Chemoprevention

Although many single compounds have potential benefits, their chemopreventive efficacy in clinical trials has been modest (about 20%) and/or they have an unacceptable toxicity. Combining low doses of different agents may be effective in increasing their efficacy while minimizing toxicity. A prospective, randomized, placebo-controlled clinical trial of combination difluoromethylornithine (DFMO) (500 mg/d), a selective inhibitor of polyamine synthesis, and sulindac (150 mg/d), a nonsteroidal anti-inflammatory drug, found that the 3-year treatment was associated with a 70% reduction of recurrence of all adenomas, and over a 90% reduction of recurrence of advanced and/or multiple adenomas, without evidence of serious toxicities⁴¹. The magnitude of the benefit in this trial was larger than expected for any type of chemoprevention.

Conclusion

Despite recent advances in medicine, the mortality from colorectal cancer, a leading cause of death in the Western countries, still remains unacceptably high. Therefore, the search for strategies to prevent the development and progression of colorectal cancer has greatly intensified. Chemoprevention offers a viable option to block neoplastic inception or delay disease progression. Despite much effort over two decades, no chemopreventive intervention for CRC has yet entered routine clinical practice as part of usual care for healthy members of the general population. The ideal chemopreventive agent remain to be discovered, with great emphasis on need not to harm. Interestingly, agent combinations may maximize effectiveness while limiting drug toxicity, using low, safe doses of two or more agents, each targeting a different cellular pathway. Finally, any strategy of chemoprevention should be always combined with screening and surveillance colonoscopy.

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