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Minireview

The Evolving Role of Nonsteroidal Anti-Inflammatory Drugs in Colon Cancer Prevention: A Cause for Optimism

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ABSTRACT

Colorectal cancer (CRC) is a serious yet preventable disease. The low acceptance and cost of colonoscopy as a screening method for CRC make chemoprevention an important option. Nonsteroidal anti-inflammatory drugs (NSAIDs), not currently recommended for CRC prevention, have the potential to evolve into the agents of choice for this indication. Here, we discuss the promise and challenge of NSAIDs for this chemopreventive application. Multiple epidemiologic studies, randomized clinical trials (RCTs) of sporadic colorectal polyp recurrence, RCTs in patients with hereditary colorectal cancer syndromes, and pooled analyses of cardiovascular-prevention RCTs linked to cancer outcomes have firmly established the ability of conventional NSAIDs to prevent CRC. NSAIDs, however, are seriously limited by their toxicity, which can become cumulative with their long-term administration for chemoprevention, whereas drug interactions in vulnerable elderly patients compound their safety. Newer, chemically modified NSAIDs offer the hope of enhanced efficacy and safety. Recent work also indicates that targeting earlier stages of colorectal carcinogenesis, such as the lower complexity aberrant crypt foci, is a promising approach that may only require relatively short use of chemopreventive agents. Drug combination approaches exemplified by sulindac plus difluoromethylornithine appear very efficacious. Identification of those at risk or most likely to benefit from a given intervention using predictive biomarkers may usher in personalized chemoprevention. Agents that offer simultaneous chemoprevention of diseases in addition to CRC, e.g., cardiovascular and/or neurodegenerative diseases, may have a much greater potential for a broad clinical application.

Introduction

Colorectal cancer (CRC) is a serious yet preventable disease. Its incidence, mortality, and financial burden to society make CRC an important health care issue. The natural history and clinical features of CRC have largely dictated our current approaches to its prevention (Tarraga Lopez et al., 2014). The long and often asymptomatic premalignant (and early malignant) stage of CRC, which can be detected and treated effectively, has provided the impetus for screening methods, with colonoscopy being the most prominent among them. Optical (as opposed to virtual) colonoscopy has the advantage of providing the cure (polypectomy) during the diagnostic session (Nishihara et al., 2013). Unfortunately, despite sophisticated national campaigns to raise awareness among the general public in the United States, the frequency of routine screening of eligible individuals continues to be low (Klabunde et al., 2011). An alternative approach to CRC prevention is chemoprevention, defined as the administration of natural or pharmacological agents to individuals at risk for CRC to prevent the development of the disease or its recurrence (Cooper et al., 2010). Nonsteroidal anti-inflammatory drugs (NSAIDs) are the best studied chemopreventive agents for CRC and the subject of the present review.

Conventional NSAIDs and Their Limiting Safety Profile

NSAIDs represent a group of over 20 drugs that enjoy broad clinical application. The first NSAID was aspirin. Synthesized by Felix Hoffmann in 1897, aspirin remains one of the most widely used medications in the world, with 40,000 tons of it being consumed each year, despite its initial rejection by Bayer as a "product [that] has no value" (Miner and Hoffhines, 2007). The vigorous development of NSAIDs reflects the great clinical need they address: the control of pain and fever, two common manifestations of a broad spectrum of diseases. Their

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ABBREVIATIONS: ACF, aberrant crypt foci; COX, cyclooxygenase; CRC, colorectal cancer; DFMO, difluoromethylornithine; DM, des-methyl; FAP, familial adenomatous polyposis; GI, gastrointestinal; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; PC, phosphatidylcholine; PGE_2, prostaglandin E_2; PS, phospho-sulindac; RCT, randomized clinical trial.

anti-inflammatory properties only served to intensify these development efforts.

Structurally, NSAIDs belong to seven diverse chemical classes: salicylates; fenamates; para-aminophenol, acetic acid, enolic acid, and propionic acid derivatives; and diaryl heterocyclic or cyclooxygenase (COX) 2–selective NSAIDs. On the basis of their ability to inhibit isozymes of COX, their best recognized molecular target, NSAIDs are classified as *nonselective*, which inhibit to a significant degree both COX-1 and COX-2, and *selective* (also called COX-2 inhibitors), which inhibit COX-2; at sites of inflammation there is more COX-2 than COX-1, which is normally present in the stomach, platelets, and blood vessels.

As with all medications, two properties of NSAIDs bear heavily on their potential role in chemoprevention: efficacy and safety. Safety is particularly important in chemoprevention, as the intake of the NSAID will be prolonged, thus increasing the possibility of side effects. There are no safe NSAIDs; in fact, their side effects are not trivial and can even be lethal (Thun and Blackard, 2009; Salvo et al., 2011). The main side effects of NSAIDs include gastrointestinal (GI) and renal toxicity (the two most important), cardiovascular, central nervous system, and platelet side effects. Although the main side effects of NSAIDs are shared, probably reflecting some commonality in their mechanism of action, there are no large scale comparative studies assessing which, if any, of the available NSAIDs is the safest. Nor are there available comparisons factoring in their relative efficacy and relative safety; such studies would address the most difficult clinical decisions in this setting but their methodological and logistical challenges are enormous.

The greatest amount of safety data for an NSAID is available for aspirin. This is not surprising, since aspirin has been available the longest and its therapeutic applications cover a wide spectrum of clinical entities. Furthermore, the long-term use of aspirin in the prevention of coronary events mirrors the envisioned application of NSAIDs in the prevention of CRC, at least in terms of its duration and perhaps the starting age of administration.

The GI side effects of aspirin have been thoroughly evaluated using several systematic reviews that included data from randomized control trials (RCTs), cohort studies, case-control studies, and some that considered low and high doses of aspirin (Dube et al., 2007). Aspirin consistently increased the risk for GI bleeding (1.6–3.1 times increased relative risk compared with those who did not use aspirin), and it also increased the risk for adverse GI symptoms, such as nausea and dyspepsia (odds ratio, 1.7) (Roderick et al., 1993). Aspirin-induced GI toxicity was both dose and age dependent (increased with increasing dose, and in older patients) (Serebruany et al., 2004).

Anyone who is at risk for or who has cardiovascular disease (coronary artery disease) may have a further increase in risk of heart attacks when taking an NSAID. Indeed, cardiac toxicity during CRC chemoprevention trials was the main reason for withdrawing COX-2–specific inhibitors (Solomon et al., 2006).

An often overlooked aspect of the safety of NSAIDs concerns their interactions with other medications (Verbeeck, 1990; Delaney et al., 2007). These interactions are particularly germane to their envisioned chemopreventive application, which will include elderly patients who may have comorbidities requiring additional medications and be more vulnerable to drug side effects on the basis of age alone. Relevant examples are the increased risk of bleeding in patients using anticoagulants, such as warfarin (Coumadin; Bristol-Myers Squibb, New York, NY) (Chan, 1995) or heparin, concurrently with NSAIDs, and the increase in phenytoin (Dilantin; Pfizer, New York, NY) blood level by NSAIDs, necessitating its monitoring when the NSAID dose is started or changed (Kaminski et al., 1998). A recent study further revealed the complex interactions between NSAIDs and several commonly used drugs, such as corticosteroids, aldosterone antagonists, and selective serotonin reuptake inhibitors, in enhancing the risk of upper GI bleeding when used in combination (Masclee et al., 2014). Underscoring the notion of cumulative toxicity, taking a second NSAID at the same time clearly increases the risk of side effects.

These and similar findings with the toxicity of NSAIDs create a conundrum, not infrequent in clinical therapeutics, that requires weighing the risk and benefit of an intervention. In the case of CRC prevention, the bar for NSAIDs (and any agent that is not totally harmless) is very high. The chemopreventive agent will often be prescribed to healthy subjects at risk for CRC who will take this agent for the rest of their lives to prevent a cancer they may never develop. Indeed, the probability that an individual at average risk will develop CRC in a given year is $<5\%$ to age 79 years (Burt et al., 1995). The obvious corollary is that in the general population about 95% of those treated to prevent CRC will not benefit from this treatment. The risk of CRC is much higher in genetically susceptible subgroups, but they only represent a small fraction of CRC (Lynch et al., 1993; Ponz de Leon et al., 1993; Burt et al., 1995). For example, the lifetime risk is 17% for those with two affected first-degree relatives, 70% for individuals with hereditary nonpolyposis colon cancer mutations, and $>95\%$ in patients with familial adenomatous polyposis (FAP). Thus a promising candidate agent should not only have an acceptable low toxicity but its efficacy should be very high, at least for those at average risk.

The Chemopreventive Efficacy of Conventional NSAIDs

The four most relevant questions in assessing the chemopreventive efficacy of conventional NSAIDs are: Are they really efficacious? If yes, are the various clinical subgroups of CRC differentially affected by NSAIDs? What is the optimal dosing? And, for how long should NSAIDs be administered for optimal chemoprevention? These questions are addressed below. As with the side effects of NSAIDs, most of the reported efficacy studies concern aspirin, the prototypical NSAID.

That conventional NSAIDs can prevent CRC is by now considered firmly established (Rostom et al., 2007; Bosetti et al., 2012; Chan et al., 2012). The evidence comes from a constellation of sources; what makes it compelling is that, with minor, largely explainable exceptions these results are impressively consistent. They include multiple epidemiologic studies that followed the path-breaking observation of Kune et al. (1988); RCTs of sporadic colorectal polyp recurrence; RCTs in patients with hereditary colorectal cancer syndromes; and pooled analyses of cardiovascular-prevention RCTs linked to cancer outcomes.

Numerous epidemiologic studies in diverse populations (and enormous in number of subjects) revealed that sustained use of NSAIDs is associated with 30–50% reduction in adenomatous polyps, incident disease, and death from CRC (Rostom et al., 2007; Thun and Blackard, 2009; Garcia-Albeniz and Chan, 2011). Prompted by these findings, several RCTs assessed the ability of aspirin to prevent the development of colon adenomas. Adenomas, the precursors of most CRCs, were selected as surrogate endpoints

for CRC prevention to shorten the period of observation to about 2 years from around the 10 years that would be required for CRC development. Although the dose of aspirin and other aspects of these RCTs varied, the efficacy of aspirin was essentially consistent, providing a modest 17–35% reduction in risk of recurrent adenoma or carcinoma (Baron et al., 2003; Benamouzig et al., 2003; Sandler et al., 2003). These results have been confirmed in Asian patients as well (Ishikawa et al., 2014).

A far more encouraging result has been reported by Gerner and Meyskins, who have diligently pursued for years a combination approach, reasoning that a second agent could bolster the real but modest effect of NSAIDs (Laukaitis and Gerner, 2011). Appreciating the role of polyamines in colonocyte proliferation, they attacked two steps of their linear biosynthetic pathway. Difluoromethylornithine (DFMO) decreases polyamine synthesis by inhibiting ornithine decarboxylase, and sulindac increases cellular export of polyamines by activating the spermidine/spermine acetyltransferase. In a phase III trial in patients with prior colon polyps, the combination of oral DFMO and sulindac reduced total metachronous colorectal adenomas by 70% and advanced and/or multiple adenomas by >90% (Meyskens et al., 2008). This regimen was well tolerated; its modest ototoxicity allayed concerns about DFMO's auditory side effects. Combination studies of aspirin and folate, however, did not produce any appreciable benefit from folate (Logan et al., 2008).

Niitsu and Takayama's groups have taken an innovative approach to CRC prevention. They have focused on aberrant crypt foci (ACF), minute mucosal lesions recognizable by magnifying endoscopy and considered precursors of polyps (Takayama et al., 1998, 2005; Rasheed and Rigas, 2008). A 12-month interim analysis of an RCT of sulindac (300 mg daily) and the COX-2 inhibitor etodolac (400 mg daily), each administered for 2 months, revealed that sulindac (but not etodolac) was able to reduce ACF at 2 months and polyps at 12 months (Takayama et al., 2011). That this effect was similar to that of NSAIDs administered long-term in trials using polyp recurrence as a surrogate marker raises critical points regarding agent selection, timing, dose, and duration of administration. Short-term and even discontinuous administration of selected NSAIDs may be all that is needed to prevent CRC; sulindac seems to be a promising candidate. More importantly, these data suggest that instead of polyps the appropriate target is ACF, low-complexity early lesions that are perhaps easier to eliminate with a small molecule like sulindac or one of its derivatives, e.g., phospho-sulindac (Mackenzie et al., 2010). A direct implication of these findings, if confirmed, is that agent administration ought to be started early, before polyps form.

Two genetic syndromes underlying familial CRC are FAP and the Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer. RCTs have demonstrated that sulindac and the COX-2–selective inhibitors celecoxib and rofecoxib reduce the size and number of colorectal polyps after 6–9 months of treatment in FAP patients (Giardiello et al., 1993; Steinbach et al., 2000; Hallak et al., 2003). Aspirin at the relatively high dose of 600 mg/day failed to significantly reduce the number of polyps in the sigmoid colon and rectum (Burn et al., 2011a). Lower doses of aspirin (100 mg/day) in FAP patients, explored in Japan, showed some efficacy but the trial was largely inconclusive owing to the small size of subgroups (Ishikawa et al., 2013).

In Lynch syndrome, aspirin 600 mg/day for up to 4 years was effective in preventing CRC (Burn et al., 2011b). For those taking aspirin for 2 years or longer the hazard ratio was 0.41 ($P = 0.02$); efficacy was lacking in those taking aspirin for <2 years. This trial, the first RCT of aspirin with CRC as the primary endpoint, provides clear evidence that aspirin is an effective chemopreventive agent in hereditary cancer with an effect equivalent to that achieved with surveillance colonoscopy. A remarkable finding was the delayed protection by aspirin against cancer.

This delayed effect of aspirin was documented in a study that pooled individual patient data and examined the effects of randomized aspirin treatment on all cancer mortality (Rothwell et al., 2011). Data from eight cardiovascular-prevention RCTs of daily aspirin were included. Administration of aspirin 75–1200 mg/day was associated with 21% lower risk of death from any cancer, but the benefit was only apparent after 5 years of follow up. The risk of death attributable to CRC was also reduced (HR = 0.41; $P = 0.05$), beginning 5 years after the initiation of aspirin treatment. Another study by the same group, which analyzed 51 RCTs of daily low-dose aspirin for primary prevention, revealed that aspirin reduced cancer incidence from 3 years onwards (Rothwell et al., 2012).

Mechanism of Action of NSAIDs. The effect of NSAIDs in cancer prevention is pleiotropic (Shiff and Rigas, 1999a,b; Kashfi and Rigas, 2005; Schror, 2011; Stolfi et al., 2013). There are at least three major classes of effects: those mediated through COX inhibition, COX-independent effects, and effects on colon stem cells. The major difficulty in evaluating potential mechanisms of action is how to integrate multiple studies into a cohesive outline. This difficulty stems often from our inability to distinguish primary proximal effects from dependent signaling changes.

There is a significant body of data indicating that COX inhibition by NSAIDs has a role in prevention of CRC (Schror, 2011); after all, COX is the best studied molecular target of aspirin and all other NSAIDs. CRC has increased levels of prostaglandin E_2 (PGE_2) (Rigas et al., 1993), which can stimulate the proliferation of CRC cells (Qiao et al., 1995), an effect blocked by NSAIDs (Shiff et al., 1996). A key role in multiple signaling loops seems to be played by NSAID-activated gene-1 (NAG-1) (Iguchi et al., 2009) and sphingosine-1 kinase (Ponnusamy et al., 2010), both of which mediate the induction of COX-2 and affect a host of relevant targets. On the other hand, there is evidence just as strong indicating that COX-independent effects also play a role in the chemopreventive effect of NSAIDs (Hanif et al., 1996) both in the colon and elsewhere. Nuclear factor-κB, the Wnt pathway, and the DNA mismatch repair system seem to mediate COX-independent effects. The notion that (at least modified) NSAIDs act by inducing a state of oxidative stress has been proposed (Rigas and Sun, 2008); interestingly, oxidative stress leads to COX-2 overexpression (Sun et al., 2009).

Authors assessing these reports take issue with the extrapolations of in vitro data to animals and then to humans or argue that studies using NSAID concentrations not encountered in vivo are not valid. It seems to us that neither concern is entirely valid (Wong et al., 2012a). What is needed is the (laborious) validation of preclinical findings in humans; in its absence, promising results should be viewed as simply prompting us to perform the definitive studies.

In recent years, colon cancer stem cells along with the tumor microenvironment have become a major focus in our efforts to understand the chemopreventive efficacy of NSAIDs (Kim, 2014). Experimental data suggest that a primary mode of direct

chemopreventive action of aspirin and other NSAIDs, such as sulindac, might be the selective induction of apoptosis in human intestinal stem cells with aberrant Wnt signaling (Qiu et al., 2010). Interestingly, the anti–colon cancer stem cell effect of NSAIDs is mediated through both COX-dependent and -independent pathways (Moon et al., 2014). Paraskeva has elegantly linked PGE_2 with colon adenoma and carcinoma stem cells by showing that the former promotes the survival of the latter (Al-Kharusi et al., 2013). Although it appears that the definitive mechanism by which NSAIDs prevent CRC is far from complete, it is fair to state that its broad outlines have been identified and a deeper understanding should be forthcoming.

Newer, Nonconventional NSAIDs. Despite the impressively consistent results summarized above, it is clear that the efficacy of NSAIDs is not optimal. Responding to the need for more potent and safer NSAIDs, several investigators have developed alternatives, all currently in preclinical or early clinical stages. In all cases, NSAIDs have been chemically modified to create new chemical entities (Table 1).

Nitric oxide–releasing NSAIDs represent a major milestone in our efforts to improve NSAIDs. They were designed to harness the then newly discovered pharmacological power of nitric oxide (NO), which could in theory abrogate the ulcerogenic properties of NSAIDs (Rigas, 2007b). To this end, a moiety that releases NO ($–ONO_2$) was covalently added to the conventional NSAID through its carboxylic group (nearly all NSAIDS are carboxylic acids). Nitroaspirin, the most extensively studied, showed significant gastroprotection and efficacy in CRC chemoprevention (Williams et al., 2004). Although it is doubtful whether the release of NO is relevant to either their safety or efficacy when administered systemically (Rigas and Williams, 2008), these compounds displayed interesting pharmacological properties (Rigas, 2007a) but their clinical assessment for CRC chemoprevention was suspended because of potential genotoxicity.

Piazza and colleagues have successfully chemically modified sulindac (Haanen, 2001; Tinsley and Piazza, 2012). For example, their novel sulindac derivative, sulindac benzylamine, does not inhibit COX-1 or COX-2, yet potently inhibits the growth and induces the apoptosis of human colon tumor cells (Whitt et al., 2012). The basis for this activity appears to involve cyclic guanosine 3′,5′,-monophosphate phosphodiesterase; the PDE5 isoform is essential for colon tumor cell growth. An outgrowth of this work is their efforts to develop a series of PDE5 inhibitors (Tinsley and Piazza, 2012).

Lichtenberger's team has associated several NSAIDs with phosphatidylcholine (PC). In rodent model systems and pilot clinical trials these PC-NSAIDs protected against GI side effects by preventing a decrease in the hydrophobic characteristics of the intestinal mucus gel layer, at the same time preserving or enhancing the therapeutic activity (Lim et al., 2013). The mucosa of the GI tract exhibits hydrophobic properties that protect the underlying epithelium from gastric acid. These characteristics appear to be attributable to an extracellular lining of surfactant-like phospholipids on the mucus gel layer; PC is the most abundant of the gastric phospholipids. Interestingly, these compounds possess full COX-inhibitory activity.

PC-NSAIDs may possess significant chemopreventive action. For example, both PC-aspirin and PC-ibuprofen directly inhibited the growth of colon cancer cells in vitro and significantly reduced the development of colonic ACF in azoxymethane-treated rats, suggesting their potential utility in patients at risk for CRC (Lichtenberger et al., 2014).

Marnett's group pursued the esterification/amidation of the carboxylic acid moiety in various NSAIDs (Kalgutkar et al., 2000), e.g., indomethacin (Kalgutkar et al., 2005), to obtain selective COX-2 inhibitors. Evaluation by the same group of several sulindac derivatives [des-methyl (DM)-sulindac sulfide and its prodrug DM-sulindac] that do not inhibit COX-2 activity revealed that only sulindac significantly inhibited tumor formation in *APC/Min* mice (Wang et al., 2011). This was attributed to conversion of DM-sulindac to DM-sulindac sulfide (active form), which was less efficient than the conversion of sulindac to its active form, sulindac sulfide, in the mice.

Phospho-NSAIDs are the most recent class of chemically modified NSAIDs to be evaluated in the prevention of CRC. Nearly all of them consist of a conventional NSAID to which a diethylphosphate moiety is added through a linker. Phosphosulindac (PS; OXT-328), much more potent than sulindac in inhibiting the growth of cultured CRC cells, showed significant chemopreventive efficacy in vivo (Mackenzie et al., 2010, 2011). In addition, PS synergized with DFMO to prevent CRC, reducing tumor multiplicity in *APC/Min* mice by 90%. Mechanistically, PS increased the intracellular levels of reactive oxygen species, key early mediators of its chemopreventive effect. Moreover, PS induced spermidine/spermine acetyltransferase enzymatic activity, and together with DFMO it reduced polyamine levels in vitro and in vivo. However, it appears that the PS/DFMO mechanism of action extends beyond polyamines and includes the thioredoxin system (Mackenzie et al., 2011), an emerging regulator of chemoprevention (Sun and Rigas, 2008).

In animal studies, the safety of PS was equivalent to that of placebo (Mackenzie et al., 2010). The remarkable safety of PS (far superior to that of sulindac) is largely explained by its unique pharmacokinetic properties: its blood area under the curve concentration from 0 to 24 hours is around 40% of that of sulindac, and in the stomach, the organ most affected by NSAID toxicity, PS is present mainly intact with minimal levels of its harmful metabolites, sulindac and sulindac sulfide (Xie et al., 2012a). PS's more rapid detoxification by cytochrome P450s and flavin monooxygenases seems to contribute to its safety (Xie et al., 2012b).

TABLE 1
Nonconventional NSAIDs

Compound or Class of Compounds	Example
Nitric oxide–releasing NSAIDs	Nitro-aspirin (NCX-4016)
NSAIDs with phosphatidylcholine	PC-aspirin
Esterified/amidated NSAIDs	DM-sulindac
Phospho-NSAIDs	Phospho-sulindac (OXT-328)
Pegylated phospho-NSAIDs	Pegylated phospho-ibuprofen

An interesting phospho-NSAID is the recently reported pegylated derivative of phospho-ibuprofen (Mattheolabakis et al., 2014). Polyethylene glycol was covalently attached to phospho-ibuprofen, known to inhibit colon cancer growth (Xie et al., 2011), to abrogate its hydrolytic degradation by esterases; many phospho-NSAIDs are carboxylic esters hydrolyzable by carboxylesterases (Wong et al., 2012b). The pegylated derivative, very resistant to hydrolysis in vivo, proved to be efficacious in CRC prevention in *APC/Min* mice and safe. Additional phospho-NSAIDs have shown significant anti-CRC activity; they include phospho-ibuprofen (Xie et al., 2011), phospho-tyrosol-indomethacin (Zhou et al., 2013) and others (Huang et al., 2011).

Discussion

Currently, NSAIDs are not recommended for the prevention of CRC. Although a plethora of studies support their efficacy, the lack of an official recommendation for their use for this indication is sound and reflects the unfavorable balance between risk and benefit (Gill and Sinicrope, 2005). Even if NSAIDs were much safer, if the risk of sporadic CRC is considered, it might be difficult to justify their chemopreventive use against CRC by the general population simply on the basis of cost, let alone their weak efficacy.

The work reviewed above, however, includes several critical findings that, when confirmed and properly developed, could lead to the use of NSAIDs for colon cancer prevention. The *enhanced efficacy* by a combination approach, such as that of conventional sulindac (or phospho-sulindac) with DFMO, makes the approach viable. The findings of Niitsu and Takayama (Takayama et al., 2011), again with sulindac, indicate that *limited intake* of the chemopreventive agent (months as opposed to years or lifetime) may have a lasting effect. Such a finding could drastically alter the calculus of CRC chemoprevention: compliance and economic consideration would become less daunting parameters. The huge (and justified) concern about *safety* could be overcome by the newer agents, some of which promise not only exceptional safety but also enhanced efficacy.

Rational *selection of the target population* could also contribute to making CRC chemoprevention a reality. The initial candidates for such a selection are AFP and Lynch syndrome. Although they represent a tiny fraction of CRC, they deserve the attention of investigators. Given the clinical course of these two syndromes, chemoprevention studies, in addition to their inherent value, would perhaps more easily establish the general principles and validity of the concept. The seminal work in Europe that was reviewed here is a major step in this direction. Besides these two "experiments of nature," predictive biomarkers would be extremely helpful in identifying those that could benefit from a chemopreventive agent. For example, there is evidence that NSAIDs reduce adenoma risk among women with high, but not low, urine levels of a metabolite of PGE_2 (Bezawada et al., 2014). Related to the eicosanoid pathway is the finding that the expression level of hydroxyprostaglandin dehydrogenase 15 (nicotinamide adenine dinucleotide) (15-PGDH) in normal colon mucosa may predict stronger benefit from aspirin chemoprevention (Fink et al., 2014). In this context, it is reasonable to expect a substantial contribution from molecular epidemiology, an integrative molecular and population health science that addresses the molecular pathogenesis and heterogeneity of diseases. Molecular pathologic analyses of CRC and especially its precursor lesions could facilitate personalized prevention (Lochhead et al., 2014).

The intriguing *latency* in the beneficial effect of aspirin seems to contrast with the findings with sulindac. However, these studies have different end points, and ACF represents the more proximal stage. As already mentioned, there are significant implications, not only for study design, but also for formulating a successful chemoprevention strategy. For example, would chemoprevention be more efficacious if it is started in subjects who have not advanced to adenomas? Intuitively, at least, it would be easier to arrest the process of colon carcinogenesis *early,* before it is too advanced to be controlled with any of the available agents.

The *newer NSAIDs*, chemically modified conventional NSAIDs, offer a real promise to overcome fundamental limitations of the existing paradigm. If the preclinical findings are validated by human studies, the approach to chemoprevention of CRC may be greatly simplified. Efficacy, safety, and cost may become less challenging. The ability of chemically modified NSAIDs to be combination partners may enhance chemoprevention efficacy to the level of practicality.

An often neglected parameter in considering agents for chemoprevention is the possibility of *multiple chemopreventive effects*. The protean efficacy of aspirin constitutes an excellent example. There is ample evidence that aspirin could prevent, admittedly rather weakly, multiple significant diseases, such as cancer, cardiovascular diseases, and neurodegenerative diseases, including Alzheimer's. Thus, such a multitargeted agent would dramatically favor the practicality of CRC chemoprevention. To fully develop such agents, those participating in or influencing the development of new agents should consider this aspect seriously and not be deterred by its complexity.

It appears clear that the field of CRC chemoprevention has advanced from the stage of exploration to a phase of maturity, in which properly weighed choices can be made. The transformation of the promise of NSAIDs to reality requires a deliberate approach that will accelerate the processes of discovery and clinical implementation. Supporting such an approach is our challenge.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Rigas, Tsioulias.

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