

NSAIDs and Chemoprevention

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Abstract: Several epidemiological, clinical and experimental studies established nonsteroidal anti-inflammatory drugs (NSAIDs) as promising cancer chemopreventive agents. Long-term use of aspirin and other NSAIDs has been shown to reduce the risk of cancer of the colon and other gastrointestinal organs as well as of cancer of the breast, prostate, lung, and skin. Understanding the action of NSAIDs provides substantial insights into the mechanisms by which these unique agents regulate tumor cell growth and enable better strategies for prevention and treatment. NSAIDs restore normal apoptosis and reduce cell proliferation in human adenomatous colorectal polyps, experimental colonic tumors, and in various cancer cell lines that have lost critical genes required for normal function. NSAIDs, particularly selective cyclooxygenase-2 (COX-2) inhibitors such as celecoxib, have been shown to inhibit angiogenesis in cell culture and in rodent models of angiogenesis. Exploration of the multistep process of carcinogenesis has provided substantial insights into the mechanisms by which NSAIDs modulate these events. However, unresolved questions with regard to safety, efficacy, optimal treatment regimen, and mechanism of action currently limit the clinical application of NSAIDs to the prevention of polyposis in FAP patients. Moreover, the development of safe and effective NSAIDs for chemoprevention is complicated by the potential that rare, serious toxicity may offset the benefit of treatment with these drugs given to healthy individuals who have a low risk of developing the disease. Growing knowledge in this area has brought about innovative approaches using combine actions of NSAIDs with other agents that have different modes of action. It has also led to the development of nitric oxide-releasing NSAIDs, that induce tumor cell apoptosis and compensate for COX function, as a means of increasing efficacy and minimizing toxicity. There is growing optimism for the view that full exploration of the role of NSAIDs in the prevention and treatment of epithelial cancers will serve towards reducing of mortality and morbidity from various cancers.



INTRODUCTION

In the year 2000, ~10 million incidences of malignant tumors and ~ 6.2 millions deaths from cancer occurred and the global burden of this disease continues to rise [1]. The American Cancer Society estimates that in the United States, there will be 1.34 million new cases and about 556,500 deaths due to cancer in 2003; thus, cancer is a major public health problem [2]. Elimination of risk factors as an approach for prevention of any given cancer, has so far had only limited success in reducing cancer burden. For example, it has been known for decades that tobacco smoking, excessive alcohol drinking, unhealthy dietary habits and life styles are major established risk factors for several types of neoplasia [3, 4], but to date not much progress has been made in eliminating these high risk factors. As an alternative approach, chemoprevention holds promise for both the prevention and treatment of cancer. Chemoprevention refers to the administration of chemical agents that occur naturally in foods (or have been synthesized) to help block tumor initiating and promoting events that are sequential stages of cancer development [4, 5]. Inhibition of these processes before the occurrence of malignant 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. 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 as preventive strategies. Such application is urgent for persons with precancerous lesions, and those diagnosed at early stages, and for subgroups with particular genetic susceptibility to cancer. Numerous experimental, epidemiologic, and clinical studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promise as anticancer agents. This paper briefly summarizes our current knowledge of the chemopreventive properties of non-steroidal anti-inflammatory drugs (NSAIDs) against cancer of colon and other gastrointestinal sites as well as cancer of the lung, breast, prostate, pancreas and skin. It examines how mechanistic data have been used to develop safer chemopreventive strategies. Since the revelation of antineoplastic properties of aspirin and aspirin-like compounds has revolutionized cancer research, the scientific community has recognized the potential contribution of NSAIDs to the prevention and control of cancer. Progress in the chemoprevention of colorectal cancer by NSAIDs during the past two decades has been very impressive.

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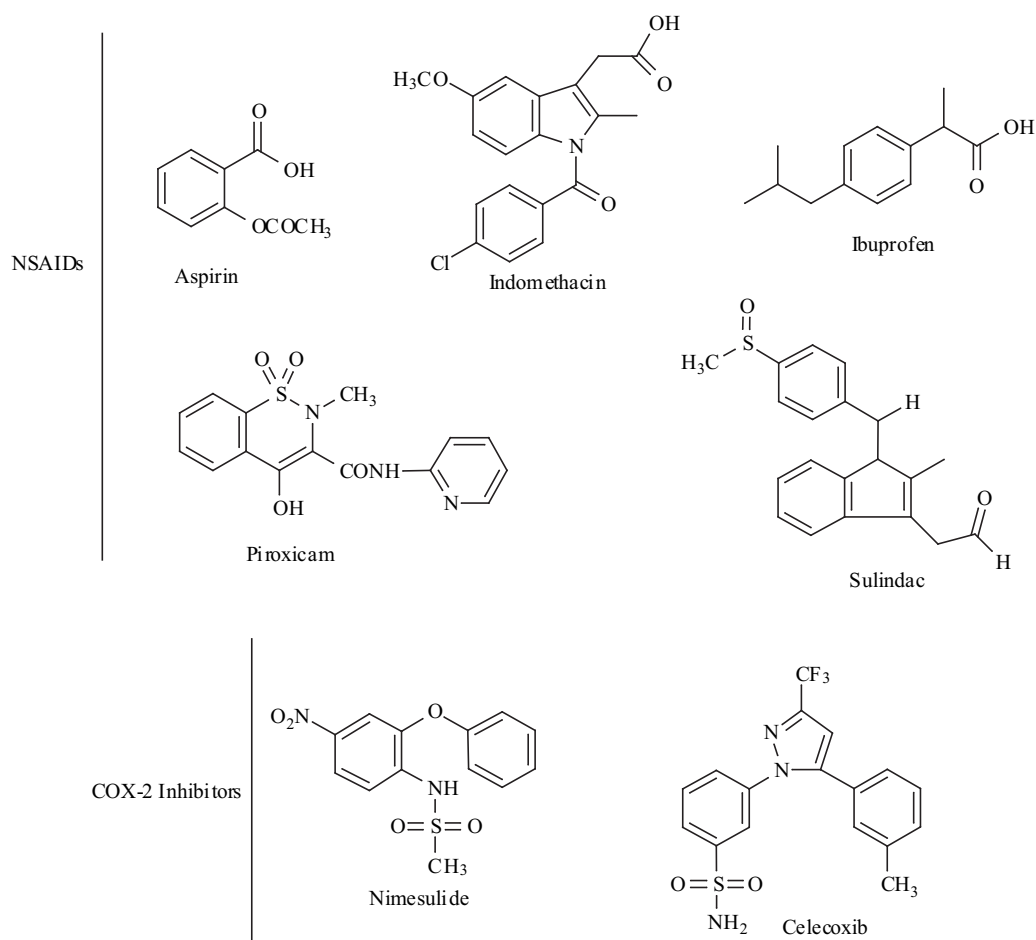


Fig. (1). Chemical structure of commonly used NSAIDs and COX-2 inhibitors

CANCER PREVENTION PROPERTIES OF NSAIDS

Epidemiologic Evidence: NSAIDs and Cancer Risk

NSAIDs are the most commonly used medication because of their pain- and fever-reducing properties. Fig. (1) shows the most frequently used NSAIDs and COX-2-selective inhibitors. Several epidemiological studies have reported that individuals who regularly use aspirin and other NSAIDs have a lower incidence of adenomatous polyps and lower incidences of or deaths from colorectal cancer compared with nonusers [6-16]. The consistency of these findings is striking, despite different researchers using various study designs in diverse patient populations. Sustained NSAIDs use is associated with a 30–50% reduction in adenomatous polyps, incident disease, and death from colorectal cancer in all but one of these studies [16]. 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 [17]. Following this investigation, several other case-control studies likewise reported a protective effect of aspirin against colorectal cancer [11, 12, 17-20].

The results of the large American Cancer Society Cancer Prevention Study of over 650,000 subjects who provided information about aspirin use, are very consistent in showing a strong inverse relationship between use of aspirin and colon cancer risk [6]. The extent of protection is similar

for both sexes and 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. Cohort studies of adenomatous colorectal polyps also indicate a significant protective effect of aspirin use and colorectal adenoma formation [9, 10]. Epidemiological studies cannot provide randomized evidence that NSAIDs prevent the development of adenomatous polyps or cancer, and to date analyses have not fully defined the optimal balance of risks and benefits in different patient populations.

Although there are fewer epidemiological studies on other cancers than on colorectal cancer, all of them have found that prolonged use of NSAIDs is associated with lower incidence of or deaths from cancers. The literature regarding these other cancers includes studies of tumors of the esophagus [7], stomach [7], breast [7, 8, 12, 16, 21-24], lung [7, 25], prostate [7, 26], urinary bladder [7], and ovaries [7, 27, 28].

Clinical Evidence: NSAIDs and FAP

Randomized clinical trials have established that the NSAIDs sulindac [29, 30] and celecoxib [31], suppress adenomatous polyps and cause regression of existing polyps in patients with FAP. FAP is a rare hereditary condition

resulting from germline inactivation of one allele of the adenomatous polyposis coli (APC) gene. Affected individuals can develop tens of thousands of adenomatous polyps. If these individuals do not undergo surgical resection of the colon, virtually all develop colorectal cancer by the third or fourth decade of life [32]. FAP accounts for only 1% of human colorectal cancers, yet it provides a model of APC inactivation as an early genetic event for the approximately 85% of cancers that develop from sporadic adenomatous polyps. Labayle *et al.* [6, 29] 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 [30]. 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. Two 400 mg doses per day of the COX-2 selective inhibitor, celecoxib, given to FAP patients for 6 months caused significant reduction of colonic polyps and size, as shown in Figure "2" [31].

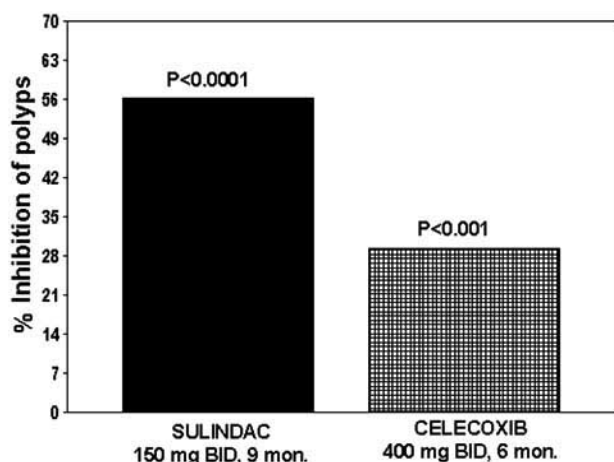


Fig. (2). Randomized clinical trials of NSAID, sulindac and COX-2 selective inhibitor, celecoxib in FAP patients. Chemopreventive effect of sulindac and celecoxib on FAP patients polyps formation based on the results of Giardiello, *et al.* (ref. [30]) and Steinbach, *et al.* (ref. [31]), respectively.

By contrast to the above observations in some of the studies some FAP patients had developed rectal carcinoma, despite ongoing therapy with sulindac [33,34] and adenomatous polyps resumed growth in FAP patients when NSAID treatment was stopped. With regard to sporadic adenomatous polyps NSAIDs 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 [35].

Experimental Evidence: NSAIDs and Chemically Induced Colon Cancer in Rodents

In the mid 1970s, Bennett and Del Tacca [36] and also Jaffe [37] observed that human colorectal tumor tissue contains high levels of prostaglandin E₂ compared to the surrounding normal mucosa. These initial observations have

paved the way to hypothesize that NSAIDs might inhibit the occurrence or growth of colorectal cancer. On the basis of this hypothesis, more than 42 experiments showed that numerous NSAIDs protect against chemically induced colorectal cancer or preneoplastic lesions formation in rats or mice [38-40, 41-61]. Pioneering studies by Pollard *et al.* [41] and Narisawa *et al.* [47] demonstrated that indomethacin and piroxicam, administered to rodents in drinking water, diet, or by *i.p.* injection inhibited colon tumors in bioassays with various carcinogens. Since then, a number of investigations have evaluated the chemopreventive efficacy of several NSAIDs against colon carcinogenesis [41-61]. In these animal model assays, weanling rats or mice were *s.c.* injected with azoxymethane or another carcinogen known to induce colon cancer and were subsequently given known concentrations of NSAIDs in their food or water. It is important to note that colorectal tumors produced in the rat model share many characteristics with human colorectal cancer, except the former have a lower tendency to metastasize [62]. The multistep nature of the rat colon carcinogenesis model provides many opportunities for intervention with agents targeted at specific mechanisms involved in the initiation, promotion, and progression of cancer. Determining the efficacy of chemopreventive 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 prevention of cancer among patients with colonic polyps. In these studies, piroxicam and sulindac were continuously administered during the promotion/progression stage of carcinogenesis, *i.e.*, after the formation of premalignant lesions in the colon. Results of these studies provided evidence that in rats piroxicam and sulindac administered 1, 14 or 24 weeks after carcinogen treatment (*i.e.*, during the promotion/progression stage) can still significantly inhibit colon tumorigenesis [39, 40]. These reports suggest that NSAIDs act to suppress colon tumor formation in rodents during both initiation and/or progression stages of colon carcinogenesis. Results generated in this preclinical model provided baseline information for eventual clinical evaluation of the efficacy of NSAIDs in the late stage intervention/prevention protocols of for high-risk individuals, such as patients with sporadic colonic polyps or FAP. In conclusion, irrespective of type of NSAIDs tested (indomethacin, aspirin, ibuprofen, piroxicam, ketoprofen and sulindac) and varying the timing of treatment (initiation/postinitiation or promotion and progression phase) these agents suppressed the incidence and multiplicity of colon tumors (Fig. (3)). The studies in rodents proved conclusively that aspirin, other conventional NSAIDs and selective COX-2 inhibitors, such as celecoxib, inhibit chemically induced carcinogenesis in rats [56] and mice [63]. In our laboratory experiments, nonselective NSAIDs typically reduced colon tumor incidence and multiplicity by 40%–70% in a dose-dependent manner, when tested at >80% of the tolerable dose levels of aspirin, ibuprofen, ketoprofen, piroxicam, and sulindac [64]. Nonselective NSAIDs suppressed but did not completely eliminate the growth of chemically induced adenocarcinomas. Our studies with celecoxib in the rat model [56, 61] have indicated that high doses of this COX-2 inhibitor (1500 ppm in food) reduced tumors by 90% and were better tolerated (~35% of tolerable

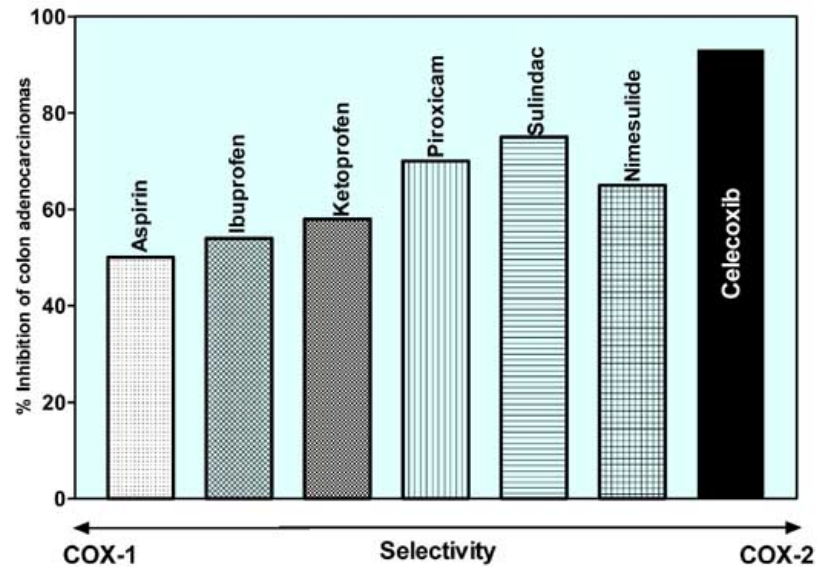


Fig. (3). Chemopreventive efficacy of NSAIDs and COX-2 selective inhibitors on AOM induced colon adenocarcinoma formation. Aspirin, ibuprofen, ketoprofen, piroxicam and sulindac were given at 80% tolerable dose and nimesulide and celecoxib were given at ~35-40% tolerable dose during initiation and postinitiation stages of colon carcinogenesis. (Ref: 153).

dose) than comparable (80% tolerable) doses of nonselective NSAIDs.

Other experimental studies have shown that NSAIDs inhibit growth of many induced and transplanted cancers in various animal models, although the evidence is less extensive than that for colorectal cancer. In line with epidemiological data, cancers at other organ sites potentially responding to NSAIDs treatment include tumors of the

esophagus*¹ (65-66), stomach [67, 68], skin [69], breast [70-73], lung [74-77], prostate [78, 79], and urinary bladder [80-82].

Experimental Evidence: NSAIDs and Mouse Models of FAP

Since the discovery and elucidation of the putative role of the APC gene as a gate-keeper gene in colon

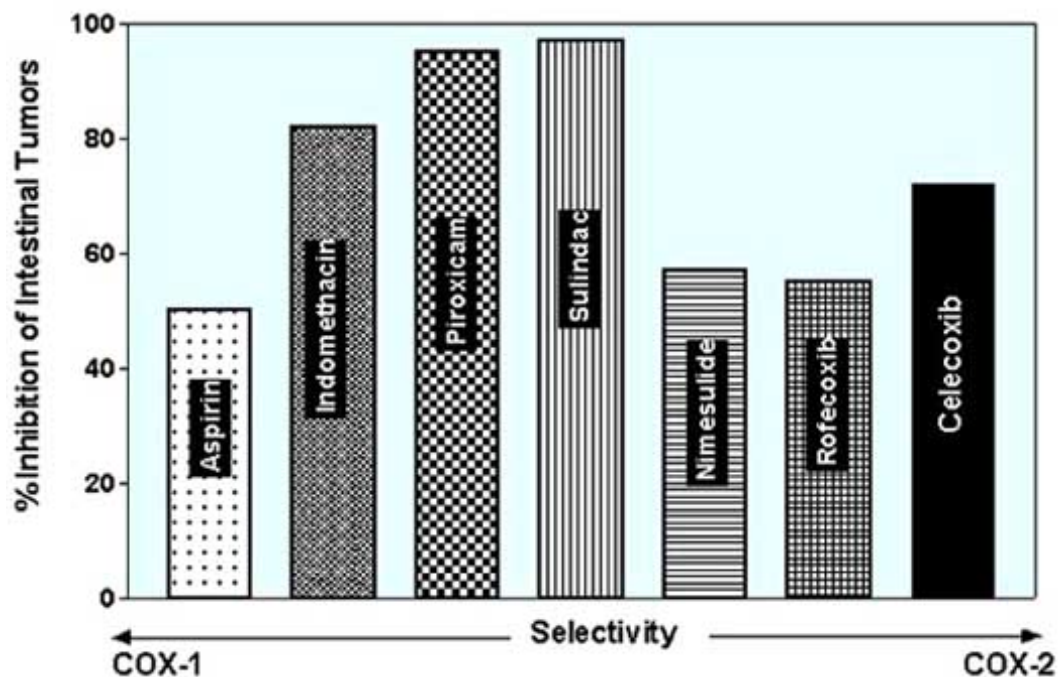


Fig. (4). The effects of various NSAIDs and COX-2 inhibitors on APCmin intestinal polyposis. In above studies NSAIDs and COX-2 inhibitors were applied various doses NSAIDs (aspirin, 800 ppm; indomethacin, 9 ppm; piroxicam, 200 ppm; sulindac, 160ppm) and COX-2 inhibitors (nimesulide, 400 ppm; celecoxib, 1500 ppm in diets and rofecoxib, 14.7 mg/Kg BW/daily) administered for 8-11 weeks. (Review: Ref. [154]).

carcinogenesis several murine models that resemble human FAP have been developed and used to determine whether various NSAIDs and COX-2 inhibitors suppress the development of spontaneous intestinal adenomas. Nonselective NSAIDs, such as piroxicam [83], sulindac [84-86], and aspirin [87], and selective COX-2 inhibitors, such as celecoxib [83], and rofecoxib [88], inhibit tumor development in *Apc^{Min}* mice and other murine models of FAP. Figure 4 summarizes effects of aspirin, sulindac, indomethacin, piroxicam, *R*-flubiprofen, nimesulide, and celecoxib on murine intestinal polyposis. 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.

MECHANISM OF ACTION OF NSAIDS

NSAIDs: Modulation of COX-Isoforms

The mechanisms by which NSAIDs act to reduce the risk of colon carcinogenesis is not yet clearly understood. Accumulating evidence points to inhibition of arachidonic acid (AA) metabolism *via* COX enzymes, which, in turn, modulate the synthesis of prostaglandins (PGs) that affect cell proliferation, tumor growth, and immune responsiveness [89, 90]. NSAIDs prevent the formation of PGH₂, the first committed step in the metabolism of AA into a complex cascade of signaling lipids, such as PGD₂, PGE₂, PGF_{2α}, PG I₂, and thromboxane B₂, the principal prostanoid metabolite in platelets (Fig. (5)). At low micromolar range (therapeutic concentrations) NSAIDs are not known to

influence other pathways of arachidonic acid metabolism except indirectly by increasing the intracellular concentration of free AA, which potentially causes shunting of AA through other metabolic pathways such as lipoxygenase [89-91].

Two mammalian isozymes, COX-1 and COX-2, encoded by different genes, are known to be present in colon tumors of humans and rodents, and to catalyze the conversion of AA to PGs [91-93]. Although COX-2 is expressed constitutively in the human kidney and brain, its expression is primarily induced in many tissues during inflammation, wound healing, and neoplasia. Increased levels of COX-2 have been found in chemically induced colon tumors in F344 rats and intestinal adenomas from *Apc^{min}* and *Apc^{Δ716}* mice [94, 95]. While 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 chiefly mediated by COX-1 [95, 97]. The expression of COX-1 does not fluctuate due to stimuli, whereas cytokines, mitogens, growth factors and tumor promoters induce COX-2 expression. COX-1 and COX-2 initiate the formation of biologically important prostanoids that coordinate signaling between the cell of origin (autocrine) and neighboring cells (paracrine) by binding to transmembrane G-protein-coupled receptors [96]. In addition, epithelial cells overexpressing the COX-2 gene develop adhesion properties and they resist undergoing apoptosis [95]. Therefore, overexpression of COX-2 may alter the proliferating capacity and tumorigenic potential of epithelial cells. In addition, it has been shown that prolonged administration of NSAIDs can cause unwanted side effects, such as gastrointestinal bleeding,

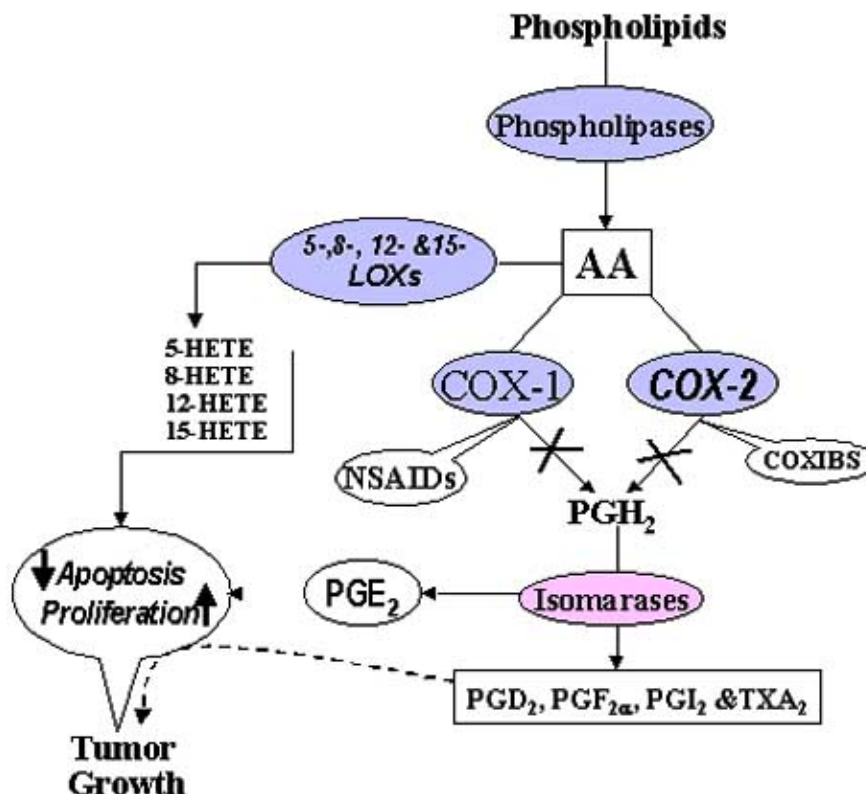


Fig. (5). Established mechanisms for NSAIDs and COX-2 inhibitors (coxibs) action on the arachidonic acid (AA) metabolism. PG, prostaglandin; HETE, hydroxyeicosatetraenoic acid. TX, thromboxane.

ulceration, and renal toxicity, which are manifested mainly by the blocking of COX-1 activity. The ability of NSAIDs to inhibit COX-1 or COX-2 varies greatly and depends on concentrations and type of tissues. Aspirin is a relatively selective inhibitor of COX-1 in platelets when given at low-doses [97, 98], but inhibits COX-2 only at plasma concentrations higher than 0.5 mM concentrations. Most other conventional NSAIDs, such as ibuprofen, piroxicam, naproxen, sulindac, and indomethacin, inhibit COX-1 and COX-2 to about the same extent, whereas the novel NSAIDs, such as celecoxib or rofecoxib selectively inhibit COX-2 [99, 100]. Thus, coxibs were developed to suppress prostanoid formation by COX-2 in inflammation and in tumorigenesis while sparing the protective effects of COX-1 and its products PG I₂ and PG E₂ on gastric epithelium.

Other studies [101, 102] argue that COX-1 activity may also be essential for the development of colorectal neoplasia. In support this, deletion of either the COX-1 or COX-2 gene in Apc-deficient mice caused a 70%–80% reduction in intestinal polyposis [101, 102]. Studies carried out in our laboratory also suggest that AOM-induced colon adenocarcinomas contain at least 3 times more COX-1 activity than does normal colonic mucosa. Importantly, in these tumor tissues the contribution of COX-1 to PG formation is equal to or more than the COX-2 contribution*². In addition, other investigators have hypothesized that COX-1 activity in activated platelets may signal the increased expression of COX-2 in other cells through the release of lipid or protein paracrine mediators [103]. A role for COX-1 either directly or in the induction of COX-2 might explain why, in epidemiological studies, aspirin use is associated with reduced risk of colorectal cancer even at doses and dosing intervals that could not sustain COX-2 inhibition in nucleated cells [6, 104].

Despite continuing disagreements about the molecular pathways by which NSAIDs may inhibit colorectal and other neoplasia, there is mounting evidence that tumor inhibition may be mediated by at least two distinct cellular processes. These involve the ability of NSAIDs to restore apoptosis in APC-deficient cells [95, 105] and their capacity, particularly in the case of COX-2 inhibitors to inhibit angiogenesis.

NSAIDs: Stimulation of Apoptosis

Programmed cell death or apoptosis, is needed to maintain homeostasis in continuously replicating tissues such as the intestine [106]. Partial suppression of apoptosis occurs early in tumorigenesis in approximately 85% of human colorectal cancers due to the inactivation of both alleles of the APC gene [107, 108]. The suppression of apoptosis allows APC-deficient cells to accumulate in adenomatous polyps. Further suppression of apoptosis occurs as these cells develop additional genetic mutations and phenotypic changes [109]. *In vitro*, both nonselective NSAIDs and selective COX-2 inhibitors stimulate apoptosis in APC-deficient cells that have not yet undergone malignant transformation. This is also seen clinically in FAP patients treated with sulindac [110] and in experimental studies of APC^{Min} mice (85, 87, 88), and rats exposed to chemical carcinogens [111]. It is important note that compared to nonselective NSAIDs, selective COX-2 inhibitors stimulate apoptosis and suppress cell proliferation

at low concentrations in cultured human cancers of the colon*³, stomach [112], esophagus [66,113], tongue [114], brain [115], lung [75], and pancreas [116].

The precise mechanism by which NSAIDs restore apoptosis and inhibit proliferation remains to be clarified [117], although it clearly affects factors related to APC deficiency or the metabolites of COX-2 or both. Manipulation of epithelial cells to overexpress COX-2 has led to suppression of apoptosis in these cells [95, 118]. Similarly, treatment of human HT-29 colon cancer cells with selective [119] or nonselective [120] COX inhibitors, or by restoring APC gene function [107], would lead to restoration of apoptosis. Recent studies from our laboratory suggest restoration of apoptosis through the modulation of p53 activity [121]. It is a fact that, COX-2 metabolites particularly electrophilic PGs do at least in part contribute to the dysfunction of p53 by impairing it in cytosol and that COX-2 selective inhibitors protect the p53 functional activity by inhibiting the electrophilic PGs [121].

NSAIDs: Modulation of COX-Independent Pathways

Despite the above described observations, results from other studies challenge the conventional wisdom that COX inhibition is the only shared function of NSAIDs [122] or that the products rather than the substrate of COX mediate its biologic effects. For example, in some experimental models, the concentration of free arachidonic acid itself regulates apoptosis in colorectal epithelial cells [123, 124]. Other experimental models suggest that NSAIDs may affect apoptosis through a mixture of prostaglandin-dependent and prostaglandin-independent pathways [117]. High concentrations of NSAIDs reportedly modify signal transduction through either the c-MYC oncogene [125], death receptors [126], nuclear factor- κ B [127], or p38, a mitogen-activated protein kinase [128]. Very high concentrations of sulindac sulfide inhibit transcriptional activation by the nuclear peroxisome proliferator-activated receptor- δ [105], a nuclear hormone receptor regulated partly by APC gene function [105,129]. NSAIDs have also been found to induce apoptosis through 15-lipoxygenase-1, independent of COX-2 [130]. However, many of these effects have been demonstrated only with high concentrations of NSAIDs *in vitro* and are of uncertain clinical relevance.

NSAIDs: Modulation of Angiogenesis

Tumors, in order to expand, must stimulate the formation of new capillary blood vessels to grow larger than approximately 2 mm in diameter [131,132]. COX-2 expression is widely induced in the angiogenic vasculature of colorectal adenomatous polyps and in carcinomas of the colon, lung, breast, esophagus, and prostate [132]. Selective COX-2 inhibitors suppress the growth of corneal capillary blood vessels in rats exposed to basic fibroblast growth factor, and inhibit the growth of several human tumors transplanted into mice [132, 133]. Therapeutic (low micromolar) concentrations of coxibs also suppress the release of angiogenic growth factors by human or rodent colorectal cancer cells that are cocultured with vascular endothelial cells [134] and block migration and tube formation by the endothelial cells. In contrast, toxic

concentrations of indomethacin [131] or aspirin [134] are required to block vascular endothelial tube formation. These experiments suggest that COX-2 may be essential for tumor vascularization and growth. However, one can argue the relevance of the experimental models to human clinical application.

Availability of COX-2 inhibitors has stimulated research on the role of COX-2 in neoplasia and the potential efficacy and safety of selective COX-2 inhibition against cancer. 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. While 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.

STRATEGIES OF IMPROVING EFFICACY OF NSAIDS

Several approaches have been developed to enhance the selectivity and reduce the toxicity of NSAIDs and specifically COX-2 inhibitors. One general approach is to identify high-risk populations in which the benefits of treatment would outweigh any of the unwarranted side effects. A second approach, which is 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 (100,

135). At present available drugs that preserve COX-1 activities are celecoxib and rofecoxib. Other highly selective COX-2 inhibitors, such as valdecoxib, etoricoxib, and COX-189, are now completing phase III trials of efficacy and safety in patients with osteoarthritis and rheumatoid arthritis. With regard to agents that compensate for COX-1 activity and/or provide protective effects against inflammation and cancer it is a valid approach to identify and develop novel NSAIDs, such as nitric oxide (NO)-NSAIDs, phytochemicals with anti-inflammatory activities, and low dose NSAIDs combined with other chemopreventive/dietary measures for the prevention of cancer.

COX-1 Sparing NO-NSAIDs

NO-NSAIDs consist of a known NSAID molecule and a nitric oxide releasing group (typically-NO₂) linked to it *via* a chemical spacer (Fig. (6)). One of the important rationales for their development was based on the observation that NO possesses several properties similar to that of 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/PGs inhibition by conventional NSAIDs. Coupling a NO-releasing moiety to a 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 [136]. *In vitro* studies with human colon cancer

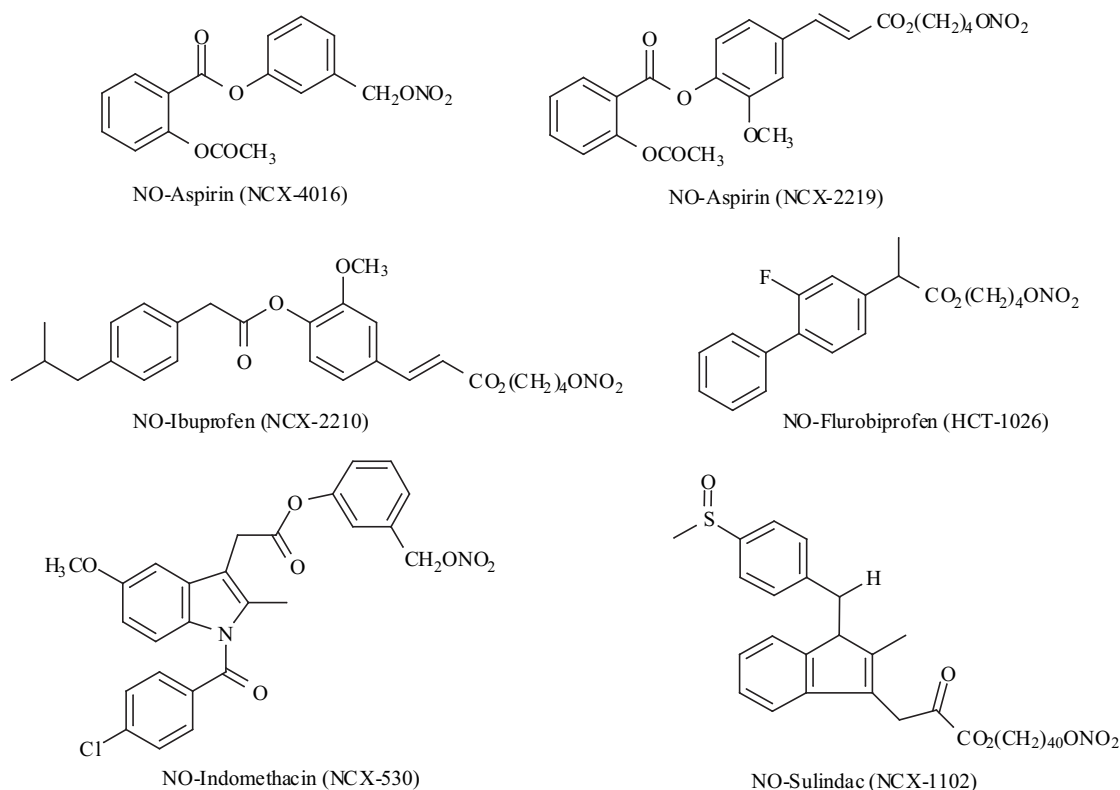


Fig. (6). NO-releasing NSAIDs, possessing COX-1 sparing activity with tumor cell inhibitory and anticarcinogenic activities (Refs. [139] and [140]).

cell lines, suggest that NO-aspirin, NO-ibuprofen and NO-sulindac are potent inducers of apoptosis. Importantly, these NO-NSAIDs are several fold more potent than the conventional NSAIDs [136]. However, the efficacy of NO-NSAIDs against the various types of cancers has been not fully established in preclinical models. Recently, we have tested a number of NO-NSAIDs for their tolerability and efficacy towards chemically induced preneoplastic lesions of the colon*⁴. In F344 rats chronic feeding of NO-aspirin, NO-flurobiprofen, NO-sulindac and NO-ibuprofen is well tolerated even at very high dose levels compared to those of their parent compounds. Importantly, these NO-NSAIDs suppressed azoxymethane-induced colonic ACF formation in the rat*⁴. At present, there are a number of studies evaluating the chemopreventive potential of NO-NSAIDs against various types of cancers. On the basis of the available *in vitro* and *in vivo* data, the NCI-sponsored a Phase II clinical trial to assess the tolerability and efficacy of NO-aspirin against ACF formation.

Phytochemicals with NSAID-Like Activities

The observation that dietary components exhibit biochemical and physiological properties analogous to NSAIDs has fostered increased interest in research on the use of such dietary substances as potential agents in reducing risk of cancer. Agents such as curcumin, phenethyl methylcaffeate, ursolic acid and oleanolic acid (Fig. (7)) have been shown to possess antiinflammatory activity*⁵ [137-140]. Importantly, most these phytochemicals induced antiinflammatory activities by modulating COX- activities similar to those induced by synthetic NSAIDs. Among naturally-occurring antiinflammatory agents curcumin was extensively studied and proven to be an inhibitor of several types of chemically induced neoplasia*⁵ [137, 139, 140]. 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 [140].

Continuous feeding of 0.2% curcumin during the initiation and postinitiation stages of AOM-induced colon carcinogenesis reduced adenocarcinoma incidence and multiplicity and the total tumor burden in F344 rats [137] Importantly, curcumin, given as a dietary supplement during the promotion/progression period, dramatically inhibited colon tumorigenesis in a dose-dependent manner [139].

Phenylethyl caffeate (PEC) and its analogue, phenylethyl methylcaffeate (PEMC) are major components of *propolis* in the honey bee hive which possesses anti-inflammatory activities and inhibits AOM-induced colonic ACF, [141] adenocarcinoma [138], DMBA-initiated/TPA-promoted skin cancer [142] and also intestinal polyp formation in APC^{min} mice [143]. Recently, we have shown that triterpenoids such as oleanolic acid and its analogues suppress COX-2 expression and activity in RAW 264.7 cells and inhibit AOM-induced colonic ACF formation in a dose manner in rats*⁵. Importantly, curcumin, phenethyl 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 antiinflammatory 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 antiinflammatory 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 phenethyl caffeate exhibit 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 these agents significantly inhibited PLA₂ and PI-PLC in the colonic mucosa and tumor tissues leading to the release of AA from phospholipids, and they

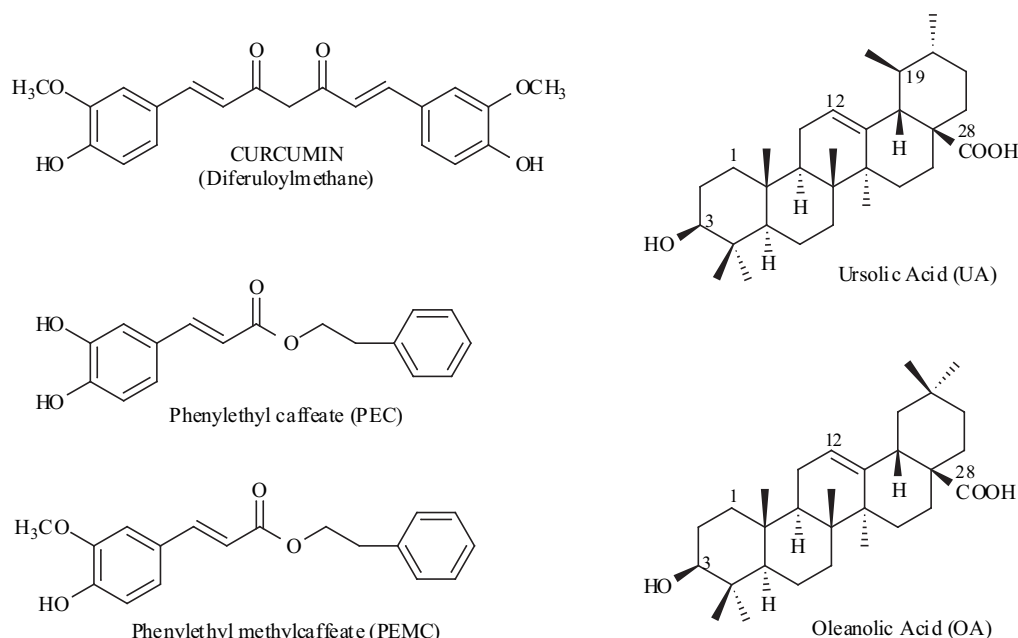


Fig. (7). Phytochemicals /Naturally-occurring agents possessing anti-inflammatory properties with potential tumor inhibitory activities.

altered COX activity, and modified PGE₂ levels [137-146]. In contrast to NSAIDs, dietary curcumin or phenethyl methylcaffate 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 [137, 138, 140]. Importantly, 12(S)-HETE is known to promote tumor cell adhesion, to stimulate the spreading of tumor cells, thus augmenting metastatic potential [144]. It is important to note that phenethyl methylcaffate preferentially suppresses the 12(S)-HETE formation in AOM-induced colonic tumors [138]. Other studies indicate that curcumin and phenethyl methylcaffate also inhibit several mediators and enzymes involved in the cell's mitogenic signal transduction pathways, and in AP-1 and NFκB activation [145, 146]. Overall, naturally-occurring antiinflammatory 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 antiinflammatory phytochemicals and NSAIDs may, in part, explain the lack of toxicity of these agents by comparison to NSAIDs.

Combinations of Low Doses of NSAIDs with other Chemopreventive Agents

Another important strategy to improve the balance of benefits and risks associated with NSAIDs use is to identify combinations of agents with different modes of action that are effective at very low doses [147, 148]. 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 piroxicam and difluoromethylornithine (DFMO), a specific, irreversibly enzyme-activated or suicide inhibitor of ornithine

decarboxylase (ODC), were evaluated for their chemopreventive efficacy. ODC-catalyzed polyamine biosynthesis plays a pivotal role in normal and neoplastic cell proliferation [39]. We have used combined administration of low 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 F344 rats (Fig. (8)). Both incidence and multiplicity of AOM-induced colon adenocarcinomas in F344 rats were significantly inhibited in F344 rats given 200 or 400 ppm piroxicam and 2000 or 4000 ppm DFMO in the diet [39]. Significantly, administration of lower doses, 100 ppm piroxicam plus 1000 ppm DFMO inhibited colon tumorigenesis even more dramatically (Fig. (8)). 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. (8)).

In other studies we have shown that much lower doses of sulindac and the cholesterol-lowering drug lovastatin are required to suppress chemically induced cancer in rodents and to stimulate apoptosis in human tumor cells when the drugs are given simultaneously than when either drug is given alone [149, 150]. Besides 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (lovastatin or lipitor), several other drugs that have been used in combination with either sulindac [147], aspirin [151], or piroxicam [38, 39]. These include ODC inhibitors [38, 39, 151], curcumin [40] and EKI-785, an irreversible inhibitor of the epidermal growth factor receptor kinase [147]. 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.

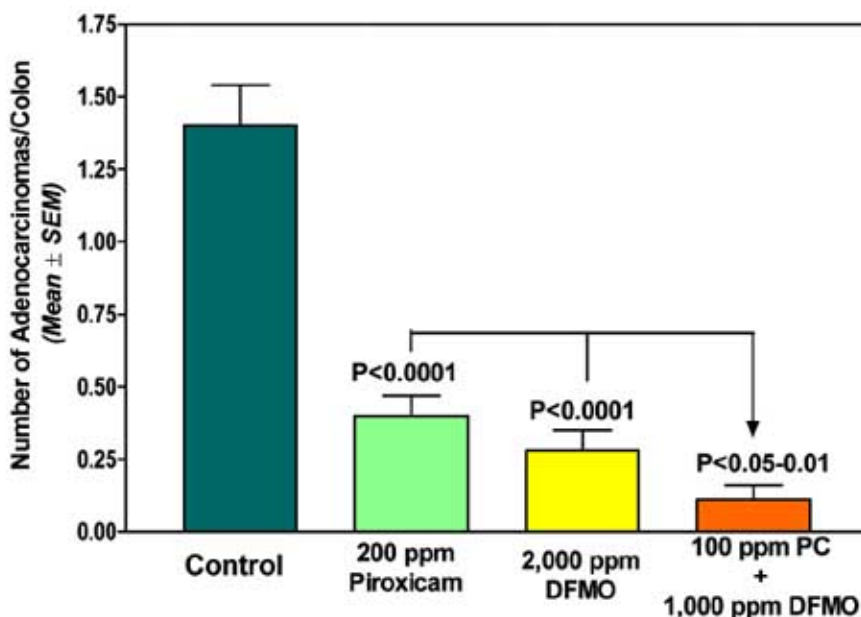


Fig. (8). Chemopreventive effect of piroxicam (NSAID) and DFMO (a ODC inhibitor) individually and in combination on AOM-induced colon adenocarcinoma formation in F344rats.

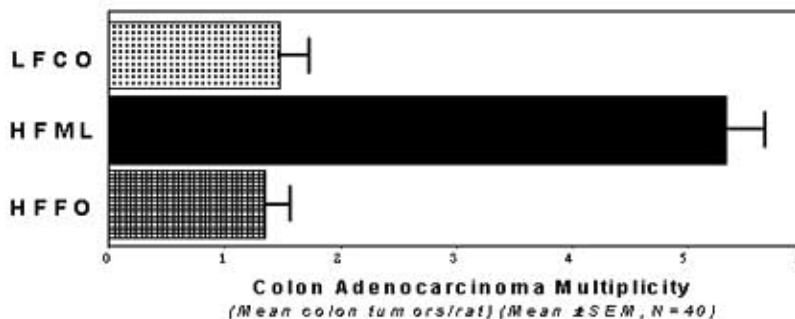


Fig. (9). Effect of types and amount of dietary fat on experimental colon adenocarcinoma formation in rat. LFCO, 5% corn oil containing diet, (routinely used for efficacy testing of NSAIDs and other chemopreventive agents); HFML, 20% mixed lipid diet, a typical Western-Style diet; and HFFO, 20% fish oil diet. (Ref: 157). Atypical US/Western-style diet is 4-to 5-fold more risk for colon adenocarcinoma formation.

Role of Diet in NSAIDs - Cancer Prevention

Based on the efficacy of NSAIDs and COX-2 inhibitors in preclinical (90%) vs clinical (~30%) applications, we believe that intervention with chemopreventive agents alone may not be sufficient for secondary prevention of cancer in high-risk patients. It has been well established that Western-style diets with high animal fat content constitute a significant risk factor for many cancers, including cancer of the colon [152]. For example, in preclinical models, we have shown that Western style diets containing 20% mixed lipids pose a 4- to 5- fold greater risk for colon cancer compared to a diet (5% corn oil) traditionally used in chemoprevention studies, or diet rich in omega-3 fatty acids (20%) (Fig. (8), [152]). These studies suggest that the high efficacy achieved in preclinical studies by administration of NSAIDs and COX-2 inhibitors with low-risk dietary strategies were not yet fully explored in clinical trials, in part resulting in lower efficacy of NSAIDs in such trials. 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. This approach is extremely important when a promising inhibitor such as celecoxib is evaluated, as it allows further improvement of its efficacy without unwanted side effects in preclinical and clinical studies.

At present, several preclinical experiments are in progress to identify novel NSAIDs and optimal combination of agents to improve efficacy and minimize the side-effects associated with gastric toxicity. In addition, more than 30 randomized clinical trials are currently testing whether nonselective NSAIDs or coxibs or combinations of low dose NSAIDs with agents that have other modes of action, can effectively treat precancerous lesions of the colon, mouth, esophagus, and skin. Moreover, such approaches are used as adjuvant therapy for solid tumors that express COX-2.

CONCLUSIONS

Numerous experimental, clinical, and epidemiological studies provide evidence that NSAIDs, particularly the highly selective COX-2 inhibitors, show promise as anticancer agents. One of the mechanisms by which NSAIDs

inhibit cancer is through the modulation of COX-1 and COX-2, isozymes that leads to a reduction of eicosanoid production, which, in turn affects cell proliferation, and apoptosis and tumor growth. Conventional NSAIDs, however, can cause unwanted side effects including gastrointestinal ulceration, bleeding, and renal toxicity, through the inhibition of constitutive COX-1 activity. Also, clinical application of NSAIDs is still limited by the lack of randomized evidence of their efficacy in populations other than those patients with FAP and adenomatous colorectal polyps. In addition, unresolved questions about the mechanism(s) by which these drugs act, the optimal drug, dose, treatment regimen, and the balance of risks and benefits in specific populations must be fully explored. Rapidly evolving progress in chemoprevention research in general has also brought about innovative approaches toward the prevention of cancer. Understanding the mechanisms at the molecular level by which NSAIDs other chemopreventive agents act offers opportunities to use combinations of specific agents. Thus, developing agents such as COX-2-selective inhibitors, NO-NSAIDs, phytochemicals with antiinflammatory activity, low-dose combinations of NSAIDs with other efficient agents, and finally combinations of low-dose NSAIDs with low-risk dietary strategies will be a practical approach for cancer prevention.

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FOOTNOTES

- *1. Carlton, P.; Gopalakrishnan, R.; Stoner, G. Expression of Cyclooxygenase-1 and Cyclooxygenase-2 in Nmba-Induced Rat Esophageal Tumorigenesis. *Proc. Am. Assoc. Cancer Res.* **1999**, *40*, 2378.

- *2. Rao, C. V.; Cooma, I.; Swamy, M. V.; Simi, B.; Reddy, B. S. Modulation of Inducible Nitric Oxide Synthase and Cyclooxygenase Activities By Curcumin During Different Stages of Experimental Colon Carcinogenesis. *Am. Assoc. For Cancer Res.* **2001**, *39*, 3084.
- *3. Malisetty, V. S.; Cooma, I.; Patlolla, J. M.; Reddy, B. S.; Rao, C. V. Chemoprevention of Colon Cancer By Modulation of Cox-2 Expression and Activity: Development of Novel Chemopreventive Regimens. *Am. Assoc. For Cancer Res.* **2003**, *41*, 4880.
- *4. Rao, C. V.; Simi, B.; Cooma, I.; Rigas, B.; Kopelovich, L.; Reddy, B. S. Chemoprevention of Colonic Aberrant Crypt Foci By Nitric Oxide (NO)-Releasing Nsaids. *Frontiers in Cancer Prevention Res.*, **2002**, *1*, Abstract D321.
- *5. Cooma, I.; Malisetty, V. S.; Patlolla, J. M.; Steele, V. E.; Rao, C. V. Chemoprevention of Colon Carcinogenesis By Oleanolic Acid and Its Analog in Male F344 Rats and Modulation of Inos and Cox-2, and Apoptosis in Human Colon Ht-29 Cancer Cells. *Am. Assoc. For Cancer Res.* **2002**, *40*, 819.

REFERENCES

- [1] World Health Organization. World Cancer Report 2003, Iarc Press, Lyon, France. **2003**.
- [2] Jemal, A.; Murray, T.; Samuels, A.; Ghafoor, A.; Ward, E.; Thun, M.J. Cancer Statistics, *Ca Cancer J. Clin.* **2003**, *53*, 5-26.
- [3] Wynder, E. L.; Kajitani, T.; Ishikawa, S.; Dodo, H.; Takano, A. Environmental Factors of Cancer of the Colon and Rectum. II. Japanese Epidemiological Data. *Cancer* **1969**, *23*, 1210-1200.
- [4] Wattenberg, L. W. Chemoprevention of Cancer. *Cancer Res.* **1985**, *45*, 1-8.
- [5] Kelloff, G. J. Perspectives on Cancer Chemoprevention Research and Drug Development. *Adv. Cancer Res.* **2000**, *78*, 1999-334.
- [6] Thun, M. J.; Namboodiri, M. M.; Heath, C. J. Jr. Aspirin Use and Reduced Risk of Fatal Colon Cancer. *N. Engl. J. Med.* **1991**, *325*, 1593-1596.
- [7] Thun, M. J.; Namboodiri, M. M.; Calle, E. E.; Flanders, W. D.; Heath, C. W. Jr. Aspirin Use and Risk of Fatal Cancer. *Cancer Res.* **1993**, *53*, 1322-1327.
- [8] Schreinemachers, D. M.; Everson, R. B. Aspirin Use and Lung, Colon, and Breast Cancer Incidence in A Prospective Study. *Epidemiology* **1994**, *5*, 138-146
- [9] Giovannucci, E.; Rimm, E. B.; Stampfer, M.; Colditz, G.; Asherio, A.; Willett, W. Aspirin Use and the Risk For Colorectal Cancer and Adenoma in Male Health Professionals. *Ann. Intern. Med.* **1994**, *121*, 241-246.
- [10] Giovannucci, E.; Willett, W. Dietary Factors and Risk of Colon Cancer. *Ann. Intern. Med.* **1994**, *26*, 443-52.
- [11] Collet, J. P.; Sharpe, C.; Belzile, E.; Boivin, J. F.; Hanley, J.; Abenhaim, L. Colorectal Cancer Prevention By Non-Steroidal Anti-Inflammatory Drugs: Effects of Dosage and Timing. *Br. J. Cancer* **1999**, *81*, 62-68.
- [12] Langman, M. J.; Cheng, K. K.; Gilman, E. A.; Lancashire, R. J. Effect of Anti-Inflammatory Drugs on Overall Risk of Common Cancer: Case-Control Study in General Practice Research Database. *Br. Med. J.* **2000**, *320*, 1642-1646.
- [13] Muscat, J.; Stellman, S. D.; Wynder, E. L. Nonsteroidal Antiinflammatory Drugs and Colorectal Cancer. *Cancer* **1994**, *74*, 1847-1854.
- [14] Greenberg, E. R.; Baron, J. A.; Freeman, D. H. Jr; Mandel, J. S.; Haile, R. Reduced Risk of Large-Bowel Adenomas Among Aspirin Users. The Polyp Prevention Study Group. *J. Natl. Cancer Inst.* **1993**, *85*, 912-916.
- [15] Peleg, I. I.; Lubin, M. F.; Cotsonis, G. A.; Clark, W. S.; Wilcox, C. M. Long-Term Use of Nonsteroidal Antiinflammatory Drugs and Other Chemopreventors and Risk of Subsequent Colorectal Neoplasia. *Dig. Dis. Sci.* **1996**, *41*, 1319-1326.
- [16] Paganini-Hill, A.; Chao, A.; Ross, R.; Henderson, B. Aspirin Use and Chronic Diseases: A Cohort Study of the Elderly. *Br. Med. J.* **1989**, *299*, 1247-1250.
- [17] Kune, G.; Kune, S.; Watson, L. F. Colorectal Cancer Risk, Chronic Illnesses, Operations, and Medications: Case Control Results From the Melbourne Colorectal Cancer Study. *Cancer Res.* **1988**, *48*, 4399-4404.
- [18] Muller, A.; Sonnenberg, A.; Wasserman, I. H. Diseases Preceding Colon Cancer: A Case-Control Study Among Veterans. *Dig. Dis. Sci.* **1994**, *39*, 2480-1484.
- [19] Reeves, M. J.; Newcomb, P. A.; Trentham-Diez, A.; Storer, B. E.; Remington, P. Nonsteroidal Anti-Inflammatory Drug Use and Protection Against Colorectal Cancer in Women. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 955-960.
- [20] Logan, R. F.; Little, J.; Hawtin, P. G.; Hardcastle, J. D. Effect of Aspirin and Non-Steroidal Anti-Inflammatory Drugs on Colorectal Adenomas: Case-Control Study of Subjects Participating in the Nottingham Faecal Occult Blood Screening Programme. *Br. Med. J.* **1993**, *307*, 285-289.
- [21] Friedman, G. D.; Ury, K. Initial Screening For Carcinogenicity of Commonly Used Drugs. *J. Natl. Cancer Inst.* **1980**, *65*, 723-733.
- [22] Egan, K. M.; Stampfer, M. J.; Giovannucci, E.; Rosner, B. A.; Colditz, G. A. Prospective Study of Regular Aspirin Use and the Risk of Breast Cancer. *J. Natl. Cancer Inst.* **1996**, *88*, 988-993.
- [23] Harris, R. E.; Kasbari, S.; Farrar, W. B. Prospective Study of Nonsteroidal Anti-Inflammatory Drugs and Breast Cancer. *Oncol. Rep.* **1999**, *6*, 71-73.
- [24] Khuder, S. A.; Mutgi, A. B. Breast Cancer and Nsaid Use: A Meta-Analysis. *Br. J. Cancer* **2001**, *84*, 1188-1192.
- [25] Muscat, J. E.; Chen, S-Q.; Richie, J. P.; Altorki, N. K.; Citron, M.; Olson, S.; Neugut, A.I.; Stellman, S. D. Risk of Lung Carcinoma Among Users of Nonsteroidal Antiinflammatory Drugs. *Cancer* **2003**, *97*, 1732-1736.
- [26] Norrish, A. E.; Jackson, R. T.; Mcrae, C. U. Non-Steroidal Anti-Inflammatory Drugs and Prostate Cancer Progression. *Int. J. Cancer* **1998**, *77*, 511-515.
- [27] Cramer, D. W.; Harlow, B.; Titus-Ernstoff, L.; Bohlke, K.; Welch, W. R.; Greenberg, E. R. Over-The-Counter Analgesics and Risk of Ovarian Cancer. *Lancet* **1998**, *351*, 104-107.
- [28] Tavani, A.; Gallus, S.; La Vecchia, C.; Conti, E.; Montella, M.; Francheschi, S. Aspirin and Ovarian Cancer: An Italian Case-Control Study. *Ann. Oncol.* **2000**, *11*, 1171-1177.
- [29] Labayle, D.; Fischer, D.; Vielh, P.; Drouhin, F.; Pariente, A.; Bories, C.; Duhamel, O.; Troussset, M.; Attali, P. Sulindac Causes Regression of Rectal Polyps in Familial Adenomatous Polyposis. *Gastroenterology* **1991**, *101*, 635-639.
- [30] Giardiello, F. M.; Hamilton, S. R.; Krush, A. J.; Piantadosi, S.; Hylind, L. M.; Celano, P.; Booker, S. V.; Robinson, C. R.; Offerhaus, G. J. Treatment of Colonic and Rectal Adenomas With Sulindac in Familial Adenomatous Polyposis. *N. Engl. J. Med.* **1993**, *328*, 1313-1316.
- [31] Steinbach, G.; Lynch, P. M.; Phillips, R. K.; Wallace, M. H.; Hawk E.; Gordon G. B.; Wakabayashi, N.; Saunders, B.; Shen, Y.; Fujimura, T; Su, L. K.; Levin, B. The Effect of Celecoxib, A Cyclooxygenase-2 Inhibitor, in Familial Adenomatous Polyposis. *N. Engl. J. Med.* **2000**, *342*, 1946-1952.
- [32] Giardiello, F. M. Nsaid-Induced Polyp Regression in Familial Adenomatous Polyposis Patients. *Gastroenterol. Clin. North Am.* **1996**, *25*, 349-362.
- [33] Lynch, H. T.; Thorson, A. G.; Smyrk, T. Rectal Cancer after Prolonged Sulindac Chemoprevention. A Case Report. *Cancer* **1995**, *75*, 936-938.
- [34] Tonelli, F.; Valanzano, R. Sulindac in Familial Adenomatous Polyposis [Letter]. *Lancet* **1993**, *342*, 1120.
- [35] Ladenheim, J.; Garcia, G.; Titzer, D.; Herzenberg, H.; Lavori, P.; Edson, R.; Omary, M. B. Effect of Sulindac on Sporadic Colonic Polyps. *Gastroenterology* **1995**, *108*, 1083-1087.
- [36] Bennett, A.; Del Tacca, M. Proceedings: Prostaglandins in Human Colonic Carcinoma. *Gut* **1975**, *16*, 409.
- [37] Jaffe, B.M. Prostaglandins and Cancer: An Update. *Prostaglandins* **1974**, *6*, 453-461.
- [38] Reddy, B. S.; Nayini, J.; Tokumo, K.; Rigottgy, K.; Zang, E.; Kelloff, G. Chemoprevention of Colon Carcinogenesis By Concurrent Administration of Piroxicam, A Nonsteroidal Antiinflammatory Drug with D,L- α -Difluoromethylornithine, An Ornithine Decarboxylase Inhibitor, in Diet. *Cancer Res.* **1990**, *50*, 2562-2568.
- [39] Rao, C. V.; Tokumo, K.; Rigotty, J.; Zang, E.; Kelloff, G.; Reddy, B. S. Chemoprevention of Colon Carcinogenesis by Dietary Administration of Piroxicam α -Difluoromethylornithine, 16- α -Fluoro-5-Androsten-17-One, and Ellagic Acid Individually and in Combination. *Cancer Res.* **1991**, *51*, 4528-4534.
- [40] Rao, C. V.; Rivenson, A.; Simi B.; Zang E.; Kelloff G.; Steele V.; Reedy, B. S.. Chemoprevention of Colon Carcinogenesis By Sulindac, A Nonsteroidal Anti-Inflammatory Agent. *Cancer Res.* **1995**, *55*, 1464-1472.

- [41] Pollard M.; Zedeck M.S. Induction of Colon Tumors in 1,2-Dimethylhydrazine-Resistant Lobund Wistar Rats By Methylazoxymethanol Acetate. *J. Natl. Cancer Inst.* **1978**, *61*, 493–494.
- [42] Pollard, M.; Luckert, P. H. Indomethacin Treatment of Rats With Dimethylhydrazine-Induced Intestinal Tumors. *Cancer Treat. Rep.* **1980**, *64*, 1323–1327.
- [43] Pollard, M.; Luckert, P. H. Effect of Indomethacin on Intestinal Tumors Induced in Rats By the Acetate Derivative of Dimethylnitrosoamine. *Science*. **1981**, *214*, 558–559.
- [44] Pollard, M.; Luckert, P. H. Prolonged Antitumor Effect of Indomethacin on Autochthonous Intestinal Tumors in Rats. *J. Natl. Cancer Inst.* **1983**, *70*, 1103–1105.
- [45] Pollard, M.; Luckert, P.H.; Schmidt, M.A. The Suppressive Effect of Piroxicam on Autochthonous Intestinal Tumors in the Rat. *Cancer Lett.* **1983**, *21*, 57–61.
- [46] Pollard, M.; Luckert, P. H. Prevention and Treatment of Primary Intestinal Tumors in Rats by Piroxicam. *Cancer Res.* **1989**, *49*, 6471–6473.
- [47] Narisawa, T.; Sato, M.; Tani, M.; Takahashi, T.; Goto, A. Inhibition of Development of Methylnitrosourea-Induced Rat Colon Tumors By Indomethacin Treatment. *Cancer Res.* **1981**, *41*, 1954–1957.
- [48] Narisawa, T.; Sato, M.; Sano, M.; Takahashi, T. Inhibition of Development of Methylnitrosourea-Induced Rat Colonic Tumors By Peroral Administration of Indomethacin. *Gann.* **1982**, *73*, 377–381.
- [49] Narisawa, T.; Satoh, M.; Sano, M.; Takahashi, T. Inhibition of Initiation and Promotion by *N*-Methylnitrosourea-Induced Colon Carcinogenesis in Rats by Non-Steroid Anti-Inflammatory Agent Indomethacin. *Carcinogenesis* **1983**, *4*, 1225–1227.
- [50] Nigro, N. D.; Bull, A. W.; Boyd, M. E. Inhibition of Intestinal Carcinogenesis in Rats: Effect of Difluoromethylornithine With Piroxicam Or Fish Oil. *J. Natl. Cancer Inst.* **1986**, *77*, 1309–1313.
- [51] Reddy, B. S.; Maruyama, H.; Kelloff, G. Dose-Related Inhibition of Colon Carcinogenesis By Dietary Piroxicam, A Nonsteroidal Antiinflammatory Drug, During Different Stages of Rat Colon Tumor Development. *Cancer Res.* **1987**, *47*, 5340–5346.
- [52] Reddy, B. S.; Tokumo, K.; Kulkarni, N.; Aliga, C.; Rao, C. V.; Kelloff, G. Inhibition of Colon Carcinogenesis By Prostaglandin Synthesis Inhibitors and Related Compounds. *Carcinogenesis* **1992**, *13*, 1019–1023.
- [53] Reddy, B.S.; Rao, C.V.; Rivenson, A.; Kelloff, G. Inhibitory Effect of Aspirin on Azoxymethane-Induced Colon Carcinogenesis in F344 Rats. *Carcinogenesis* **1993**, *14*, 1493–1497.
- [54] Moorghen, M.; Ince, P.; Finney, K. J.; Sunter, J. P.; Watson, A. J.; Appleton, D. R. The Effect of Sulindac on Colonic Tumor Formation in Dimethylhydrazine-Treated Mice. *Acta. Histochem. Suppl.* **1990**, *39*, 195–199.
- [55] Skinner, S. A.; Penney, A. G.; O'brien, P. Sulindac Inhibits the Rate of Growth and Appearance of Colon Tumors in the Rat. *Arch. Surg.* **1991**, *126*, 1094–1096.
- [56] Kawamori, T.; Rao, C. V.; Seibert, K.; Reddy, B. S. Chemopreventive Activity of Celecoxib, A Specific Cyclooxygenase-2 Inhibitor, Against Colon Carcinogenesis. *Cancer Res.* **1998**, *58*, 409–412.
- [57] Reddy, B. S.; Kawamori, T.; Lubet, R. A.; Steele, V. E.; Kelloff, G. J.; Rao, C. V. Chemopreventive Efficacy of Sulindac Sulfone Against Colon Cancer Depends on Time of Administration During Carcinogenic Process. *Cancer Res.* **1999**, *59*, 3387–3391.
- [58] Wargovich, M. J.; Chen, C.D.; Harris, C.; Yang, E.; Velasco, M. Inhibition of Aberrant Crypt Growth by Non-Steroidal Anti-Inflammatory Agents and Differentiation Agents in the Rat Colon. *Int. J. Cancer* **1995**, *60*, 515–519.
- [59] Reddy, B. S.; Rao, C. V.; Seibert, K. Evaluation of Cyclooxygenase-2 Inhibitor For Potential Chemopreventative Properties in Colon Carcinogenesis. *Cancer Res.* **1996**, *56*, 4566–4569.
- [60] Takahashi, M.; Fukutake, M.; Yokota, S.; Ishida, K.; Wakabayashi, K.; Sugimura, T. Suppression of Azoxymethane-Induced Aberrant Crypt Foci in Rat Colon by Nimesulide, A Selective Inhibitor of Cyclooxygenase 2. *J. Cancer Res. Clin. Oncol.* **1996**, *122*, 219–222.
- [61] Reddy, B. S.; Hirose, Y.; Lubet, R.; Steele, V.; Kelloff, G.; Paulson, S.; Rao, C. V. Chemoprevention of Colon Cancer By Specific Cyclooxygenase-2 Inhibitor, Celecoxib, Administered During Different Stages of Carcinogenesis. *Cancer Res.* **2000**, *60*, 293–297.
- [62] Weisburger, J.; Reddy, B.; Jofte, D. Colorectal Cancer. International Union Against Cancer (Uicc) Technical Report 19. Geneva (Switzerland): Uicc; **1975**.
- [63] International Agency for Research on Cancer (IARC). Non-Steroidal Anti-Inflammatory Drugs. In: Iarc Handbooks of Cancer Prevention. Lyon (France): IARC Press; **1997**.
- [64] Reddy, B. S.; Rao, C. V. Novel Approaches For Colon Cancer Prevention By Cyclooxygenase Inhibitors. *J. Env. Pathol. Toxicol. Oncol.* **2002**, *21*, 155–164.
- [65] Botha, J. H.; Robinson, K. M.; Ramchurren, N.; Reddi, K.; Norman, R. J. Human Esophageal Carcinoma Cell Lines: Prostaglandin Production, Biological Properties, and Behavior in Nude Mice. *J. Natl. Cancer Inst.* **1986**, *76*, 1053–1056.
- [66] Li, M.; Lotan, R.; Levin, B.; Tahara, E.; Lippman, S. M.; Xu, X. C. Aspirin Induction of Apoptosis in Esophageal Cancer: A Potential For Chemoprevention. *Cancer Epidemiol. Biomarkers Prev.* **2000**, *9*, 545–549.
- [67] Lehnert, T.; Deschner, E. E.; Karmali, R. A.; Decosse, J. J. Effect of Flurbiprofen and 16,16-Dimethyl-Prostaglandin E₂ on Gastrointestinal Tumorigenesis Induced By *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine in Rats. I. Squamous Epithelium and Mesenchymal Tissue. *J. Natl. Cancer Inst.* **1987**, *78*, 923–929.
- [68] Shibata, M. A.; Hirose, M.; Masuda, A.; Kato T.; Mutai, M.; Ito, N. Modification of Bha Forestomach Carcinogenesis in Rats: Inhibition By Diethylmaleate Or Indomethacin and Enhancement By A Retinoid. *Carcinogenesis* **1993**, *14*, 1265–1269.
- [69] Fischer, S. M.; Lo, H. H.; Gordon, G. B.; Seibert, K.; Kelloff, G.; Lubet, R. A.; Conti, C.J. Chemopreventive Activity of Celecoxib, A Specific Cyclooxygenase-2 Inhibitor, and Indomethacin Against Ultraviolet Light-Induced Skin Carcinogenesis. *Mol. Carcinog.* **1999**, *25*, 231–240.
- [70] Rozić, J. G.; Chakraborty, C.; Lala, P. K. Cyclooxygenase Inhibitors Retard Murine Mammary Tumor Progression by Reducing Tumor Cell Migration, Invasiveness and Angiogenesis. *Int. J. Cancer* **2001**, *93*, 497–506.
- [71] Liu, C.H.; Chang, S. H.; Narko, K.; Trifan, O. C.; Wu, M. T.; Smith, E.; Haudenschild, C.; Lane, T.F.; Hla, T. Overexpression of Cyclooxygenase-2 Is Sufficient To Induce Tumorigenesis in Transgenic Mice. *J. Biol. Chem.* **2001**, *276*, 18563–18569.
- [72] Alshafie, G. A.; Abou-Issa, H. M.; Seibert, K.; Harris, R. E. Chemotherapeutic Evaluation of Celecoxib, A Cyclooxygenase-2 Inhibitor, in A Rat Mammary Tumor Model. *Oncol. Rep.* **2000**, *7*, 1377–1381.
- [73] Nakatsugi, S.; Ohta, T.; Kawamori, T.; Mutoh, M.; Tanigawa, T.; Watanabe, K.; Sugie, S.; Sugimura, T.; Wakabayashi, K. Chemoprevention By Nimesulide, A Selective Cyclooxygenase-2 Inhibitor, of 2-Amino-1-Methyl-6-Phenylimidazo[4,5-*B*]pyridine (Phip)-Induced Mammary Gland Carcinogenesis in Rats. *Jpn. J. Cancer Res.* **2000**, *91*, 886–1892.
- [74] Yao, R.; Rioux, N.; Castonguay, A.; You, M. Inhibition of Cox-2 and Induction of A Apoptosis: Two Determinants of Nonsteroidal and Anti-Inflammatory Drugs' Chemopreventive Efficacies in Mouse Lung Tumorigenesis. *Exp. Lung Res.* **2000**, *26*, 731–742.
- [75] Tsubouchi, Y.; Mukai, S.; Kawahito, Y.; Yamada, R.; Kohno, M.; Inoue, K.; Sano, H. Meloxicam Inhibits the Growth of Non-Small Cell Lung Cancer. *Anti Cancer Res.* **2000**, *20*, 2867–2872.
- [76] Duperron, C.; Castonguay, A. Chemoprevention Efficacies of Aspirin and Sulindac Against Lung Tumorigenesis in A/J Mice. *Carcinogenesis* **1997**, *18*, 1001–1006.
- [77] Rioux, N.; Castonguay, A. Prevention of NNK-Induced Lung Tumorigenesis in A/J Mice by Acetylsalicylic Acid and NS-398. *Cancer Res.* **1998**, *58*, 5354–5360.
- [78] Tremblay, C.; Dore, M.; Bochsler, P.; Sirois, J. Induction of Prostaglandin G/H Synthase-2 in A Canine Model of Spontaneous Prostatic Adenocarcinoma. *J. Natl. Cancer Inst.* **1999**, *91*, 1398–1403.
- [79] Liu, X. H.; Kirschenbaum, A.; Yao, S.; Lee, R.; Holland, J. F.; Levine, A. C. Inhibition of Cyclooxygenase-2 Suppresses Angiogenesis and the Growth of Prostate Cancer in *Vivo*. *J. Urol.* **2000**, *164*, 820–825.
- [80] Okajima, A.; Denda, A.; Ozono, S.; Takahama, M.; Akai, H.; Sasaki, Y.; Kitayama, W.; Wakabayashi, K.; Konishi, Y. Chemopreventive Effects of Nimesulide, A Selective Cyclooxygenase-2 Inhibitor, on the Development of Rat Urinary

- Bladder Carcinomas Initiated By *N*-Butyl-*N*-(4-Hydroxybutyl)Nitrosamine. *Cancer Res.* **1998**, *58*, 3028–3031.
- [81] Grubbs, C. J.; Lubet R. A.; Koki, A. T.; Leahy, K. M.; Masferrer, J. L.; Steele, V. E.; Kelloff, G. J.; Hill, D. L.; Seibert, K. Celecoxib Inhibits *N*-Butyl-*N*-(4-Hydroxybutyl)-Nitrosamine-Induced Urinary Bladder Cancers in Male B6d2f1 Mice and Female Fischer-344 Rats. *Cancer Res.* **2000**, *60*, 5599–5602.
- [82] Klan, R.; Knispel, H.; Meier, T. Acetylsalicylic Acid Inhibition of *N*-Butyl-(4-Hydroxybutyl)Nitrosamine-Induced Bladder Carcinogenesis in Rats. *J. Cancer Res. Clin. Oncol.* **1993**, *119*, 482–485.
- [83] Jacoby, R. F.; Seibert, K.; Cole, C. E.; Kelloff, G.; Lubet, R. A. the Cyclooxygenase-2 Inhibitor Celecoxib Is A Potent Preventive and Therapeutic Agent in the Min Mouse Model of Adenomatous Polyposis. *Cancer Res.* **2000**, *60*, 5040–5044.
- [84] Boolbol, S. K.; Dannenberg, A. J.; Chadburn, A.; Martucci, C.; Guo, X. J.; Ramonetti, J. T.; Abreugoris, M.; Newmark, H.L.; Lipkin, M.L.; DeCosse, J.J.; Bertagnolli, M.M. Cyclooxygenase-2 Overexpression and Tumor Formation Are Blocked By Sulindac in A Murine Model of Familial Adenomatous Polyposis. *Cancer Res.* **1996**, *56*, 2556–2560.
- [85] Chiu, C. H.; Mcentee, M. F.; Whelan, J. Sulindac Causes Rapid Regression of Preexisting Tumors in Min/+ Mice Independent of Prostaglandin Biosynthesis. *Cancer Res.* **1997**, *57*, 4267–4273.
- [86] Mahmoud, N. N.; Bilinski, R. T.; Churchill, M. R.; Edelman W.; Kucherlapati R.; Bertagnolli, M. M. Genotype-Phenotype Correlation in Murine Apc Mutation: Differences in Enterocyte Migration and Response To Sulindac. *Cancer Res.* **1999**, *59*, 353–359.
- [87] Mahmoud, N. N.; Dannenberg, A. J.; Mestre, J.; Bilinski, R. T.; Churchill, M. R.; Martucci, C.; Newmark, H.; Bertagnolli, M.M. Aspirin Prevents Tumors in A Murine Model of Familial Adenomatous Polyposis. *Surgery* **1998**, *124*, 225–231.
- [88] Oshima, M.; Murai, N.; Kargman, S.; Arguello, M.; Luk, P.; Kwong, E.; Taketo, M. M.; Evans, J. F. Chemoprevention of Intestinal Polyposis in the Apc(Delta)716 Mouse By Rofecoxib, A Specific Cyclooxygenase-2 Inhibitor. *Cancer Res.* **2001**, *61*, 1733–1740.
- [89] Marnett, L. J. Aspirin and the Potential Role of Prostaglandins in Colon Cancer. *Cancer Res.* **1992**, *52*, 5575–5589.
- [90] Smith, W. L.; Dewitt, D. L.; Garavito, R. M. Cyclooxygenases: Structural, Cellular, and Molecular Biology. *Annu. Rev. Biochem.* **2000**, *69*, 145–182.
- [91] Eberhart, C. E.; Dubois, R. N. Eicosanoids and the Gastrointestinal Tract. *Gastroenterology* **1995**, *109*, 285–301.
- [92] Kargman, S. L.; O'neill, G. P.; Vickers, P. J.; Evans, J. F.; Mancini, J. A.; Jothy, S. Expression of Prostaglandin G/H Synthase-1 And-2 Protein in Human Colon Cancer. *Cancer Res.* **1995**, *55*, 2556–2559.
- [93] Dubois, R. N.; Radhika, A.; Reddy, B. S.; Entingh, A. J. Increased Cyclooxygenase-2 Levels in Carcinogen-Induced Rat Colonic Tumors. *Gastroenterology* **1996**, *110*, 1259–1262.
- [94] Taketo, M. M. Cyclooxygenase-2 Inhibitors in Tumorigenesis (Part I) *J. Natl. Cancer Inst.* **1998**, *90*, 1529–1536.
- [95] Tsujii, M.; Dubois, R. N. Alterations in Cellular Adhesion and Apoptosis in Epithelial Cells Overexpressing Prostaglandin Endoperoxide Synthase-2. *Cell* **1995**, *83*, 493–501.
- [96] Morrow, J. D.; Roberts, L. J. Lipid-Derived Autacoids. In: Hardman Jg, Limbird Le, Editors. the Pharmacological Basis of Therapeutics. New York (Ny): Mcgraw-Hill; **2001**. P. 669–685.
- [97] Patrignani, P.; Filabozzi, P.; Patrono, C. Selective Cumulative Inhibition of Platelet Thromboxane Production By Low-Dose Aspirin in Healthy Subjects. *J. Clin. Invest.* **1982**, *69*, 1366–1372.
- [98] Fitzgerald, G. A.; Oates, J. A.; Hawiger, J.; Maas, R. L.; Roberts L. J. 2nd; Lawson J.A.; Brash, A.R. Endogenous Biosynthesis of Prostacyclin and Thromboxane and Platelet Function During Chronic Administration of Aspirin in Man. *J. Clin. Invest.* **1983**, *71*, 678–688.
- [99] Peterson, W. L.; Cryer, B. Cox-1-Sparing Nsaids—Is the Enthusiasm Justified? *J. Am. Med. Assoc.* **1999**, *282*, 1961–1963.
- [100] Willoughby, D. A.; Moore, A. R.; Colville-Nash, P. R. Cox-1, Cox-2, and Cox-3 and the Future Treatment of Chronic Inflammatory Disease. *Lancet* **2000**, *355*, 646–648.
- [101] Langenbach, R.; Morham, S. G.; Tiano, H. F.; Loftin, C. D.; Ghanayem B. I.; Chulada, P.C.; Mahler, J.F.; Lee, C.A.; Goulding, E.H.; Kluckman, K.D.; Kim, H.S.; Smithies, O. Prostaglandin Synthase-1 Gene Disruption in Mice Reduces Arachidonic Acid-Induced Inflammation and Indomethacin-Induced Gastric Ulceration. *Cell* **1995**, *83*, 483–492.
- [102] Langenbach, R.; Loftin, C.; Lee, C.; Tiano, H. Cyclooxygenase Knockout Mice: Models For Elucidating Isoform-Specific Functions. *Biochem. Pharmacol.* **1999**, *58*, 1237–4126.
- [103] Patrono, C.; Patrignani, P.; Garcia Rodriguez, L. A. Cyclooxygenase-Selective Inhibition of Prostanoid Formation: Transducing Biochemical Selectivity Into Clinical Read-Outs. *J. Clin. Invest.* **2001**, *108*, 7–13.
- [104] Giovannucci, E.; Egan, K. M.; Hunter, D. J.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C.; Speizer, F.E. Aspirin and the Risk of Colorectal Cancer in Women. *N. Engl. J. Med.* **1995**, *333*, 609–614.
- [105] He, T. C.; Chan, T. A.; Vogelstein, B.; Kinzler, K. W. PPARdelta Is an Apc-Regulated Target of Nonsteroidal Anti-Inflammatory Drugs. *Cell* **1999**, *99*, 335–345.
- [106] Afford, S.; Randhawa, S. Apoptosis. *Mol. Pathol.* **2000**, *53*, 55–63.
- [107] Morin, P.J.; Vogelstein, B.; Kinzler, K.W. Apoptosis and Apc in Colorectal Tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7950–7954.
- [108] Kinzler, K. W.; Vogelstein, B. Colorectal Tumors. In: *The Genetic Basis of Human Cancer*. Vogelstein, B.; Kinzler K., Eds. New York, NY: Mcgraw-Hill; **1998**. pp. 565–587.
- [109] Bedi, A.; Pasricha, P. J.; Akhtar, A. J.; Barber, J. P.; Bedi, G. C.; Giardiello F. M.; Zehnbauer, B.A.; Hamilton, S.R.; Jones, R.J. Inhibition of Apoptosis During Development of Colorectal Cancer. *Cancer Res.* **1995**, *55*, 1811–1816.
- [110] Pasricha, P. J.; Bedi, A.; O'connor K.; Rashid, A.; Akhtar, A. J.; Zahurak, M. V.; Piantadosi, S.; Hamilton, S.R.; Giardiello, F.M. The Effects of Sulindac on Colorectal Proliferation and Apoptosis in Familial Adenomatous Polyposis. *Gastroenterology* **1995**, *109*, 994–998.
- [111] Samaha, H.; Kelloff, G.; Steele, V.; Rao, C.; Reddy, B. S. Modulation of Apoptosis By Sulindac, Curcumin, Phenylethyl-3-Methylcaffeate, and 6-Phenylhexyl Isothiocyanate: Apoptotic Index As A Biomarker in Colon Cancer Chemoprevention and Promotion. *Cancer Res.* **1997**, *57*, 1301–1305.
- [112] Uefuji, K.; Ichikura, T.; Shinomiya, N.; Mochizuki, H. Induction of Apoptosis by Jte-522, A Specific Cyclooxygenase-2 Inhibitor, in Human Gastric Cancer Cell Lines. *Anti Cancer Res.* **2000**, *20*, 4279–4284.
- [113] Souza, R. F.; Shewmake, K.; Beer, D. G.; Cryer, B.; Spechler, S. J. Selective Inhibition of Cyclooxygenase-2 Suppresses Growth and Induces Apoptosis in Human Esophageal Adenocarcinoma Cells. *Cancer Res.* **2000**, *60*, 5767–5772.
- [114] Sumitani, K.; Kamijo, R.; Toyoshima, T.; Nakanishi, Y.; Takizawa, K.; Hatori, M.; Nagumo, M. Specific Inhibition of Cyclooxygenase-2 Results in Inhibition of Proliferation of Oral Cancer Cell Lines Via Suppression of Prostaglandin E₂ Production. *J. Oral Pathol. Med.* **2001**, *30*, 41–47.
- [115] Joki, T.; Heese, O.; Nikas, D. C.; Bello, L.; Zhang, J.; Kraeft, S. K.; Seyfried, N.T.; Abe, T.; Chen, L.B.; Carroll, R.S.; Black, P.M. Expression of Cyclooxygenase 2 (COX-2) in Human Glioma and *In Vitro* Inhibition By A Specific COX-2 Inhibitor, Ns-398. *Cancer Res.* **2000**, *60*, 4926–4931.
- [116] Molina, M. A.; Sitja-Arnau, M.; Lemoine, M. G.; Frazier, M. L.; Sinicrope, F. A. Increased Cyclooxygenase-2 Expression in Human Pancreatic Carcinomas and Cell Lines: Growth Inhibition By Nonsteroidal Anti-Inflammatory Drugs. *Cancer Res.* **1999**, *59*, 4356–4362.
- [117] Marx, J. Cancer Research. Anti-Inflammatories Inhibit Cancer Growth—But How? [News] *Science* **2001**, *291*, 581–582.
- [118] Dubois, R. N.; Shao, J.; Tsujii, M.; Sheng, H.; Beauchamp, R. D. G₁ Delay in Cells Overexpressing Prostaglandin Endoperoxide Synthase-2. *Cancer Res.* **1996**, *56*, 733–737.
- [119] Elder, D. J.; Halton, D. E.; Hague, A.; Paraskeva, C. Induction of Apoptotic Cell Death in Human Colorectal Carcinoma Cell Lines By A Cyclooxygenase-2 (Cox-2)-Selective Nonsteroidal Anti-Inflammatory Drug: Independence From Cox-2 Protein. *Clin. Cancer Res.* **1997**, *3*, 1679–1683.
- [120] Shiff, S.J.; Koutsos, M.I.; Qiao, L.; Rigas, B. Nonsteroidal Antiinflammatory Drugs Inhibit the Proliferation of Colon Adenocarcinoma Cells: Effects on Cell Cycle and Apoptosis. *Exp. Cell Res.* **1996**, *222*, 179–188.

- [121] Malisetty, V. S.; Herzog, C.; Rao, C. V. Celecoxib Inhibition of COX-2 in Colon Cancer Cell Lines Increases the Nuclear Localization of Active P53. *Cancer Res.* **2003**, *63*, 5239-5242.
- [122] Vane, J. R. Inhibition of Prostaglandin Synthesis As A Mechanism of Action For Aspirin-Like Drugs. *Nat. New Biol.* **1971**, *231*, 232-235.
- [123] Cao, Y.; Pearman, A. T.; Zimmerman, G. A.; McIntyre, T. M.; Prescott, S. M. Intracellular Unesterified Arachidonic Acid Signals Apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11280-11285.
- [124] Chan, T. A.; Morin, P. J.; Vogelstein, B.; Kinzler, K. W. Mechanisms Underlying Nonsteroidal Antiinflammatory Drug-Mediated Apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 681-686.
- [125] He, T. C.; Sparks, A. B.; Rago, C.; Hermeking, H.; Zawel, L.; Da Costa, L. T.; Morin, P.J.; Vogelstein, B.; Kinzler, K.W. Identification of C-Myc As A Target of the APC Pathway. *Science* **1998**, *281*, 1509-1512.
- [126] Rice, P. L.; Goldberg, R. J.; Ray, E. C.; Driggers, L. J.; Ahnen, D. J. Inhibition of Extracellular Signal-Regulated Kinase 1/2 Phosphorylation and Induction of Apoptosis by Sulindac Metabolites. *Cancer Res.* **2001**, *61*, 1541-1547.
- [127] Yamamoto, Y.; Yin, M. J.; Lin, K. M.; Gaynor, R. B. Sulindac Inhibits Activation of the Nf-Kappab Pathway. *J. Biol. Chem.* **1999**, *274*, 27307-27314.
- [128] Schwenger, P.; Bellosta, P.; Vietor, I.; Basilico, C.; Skolnik, E. Y.; Vilcek, J. Sodium Salicylate Induces Apoptosis Via P38 Mitogen-Activated Protein Kinase But Inhibits Tumor Necrosis Factor-Induced C-Jun N-Terminal Kinase/Stress-Activated Protein Kinase Activation. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2869-2873.
- [129] Wu, G. D. A Nuclear Receptor to Prevent Colon Cancer. *N. Engl. J. Med.* **2000**, *342*, 651-653.
- [130] Shureiqi, I.; Chen, D.; Lotan, R.; Yang, P.; Newman, R. A.; Fischer, S. M.; Lippman, S.M. 15-Lipoxygenase-1 Mediates Nonsteroidal Anti-Inflammatory Drug-Induced Apoptosis Independently of Cyclooxygenase-2 in Colon Cancer Cells. *Cancer Res.* **2000**, *60*, 6846-6850.
- [131] Jones, M. K.; Wang, H.; Peskar, B. M.; Levin, E.; Itani, R. M.; Sarfeh, I. J.; Tarnawski, A.S. Inhibition of Angiogenesis By Nonsteroidal Anti-Inflammatory Drugs: Insight Into Mechanisms and Implications For Cancer Growth and Ulcer Healing. *Nat. Med.* **1999**, *5*, 1418-1423.
- [132] Masferrer, J. L.; Leahy, K. M.; Koki, A.T.; Zweifel, B.S.; Settle, S.L.; Woerner, B.M.; Edwards, D.A.; Flickinger, A.G.; Moore, R.J.; Seibert, K. Antiangiogenic and Antitumor Activities of Cyclooxygenase-2 Inhibitors. *Cancer Res.* **2000**, *60*, 1306-1311.
- [133] Williams, C. S.; Tsujii, M.; Reese, J.; Dey, S. K.; Dubois, R. N. Host Cyclooxygenase-2 Modulates Carcinoma Growth. *J. Clin. Invest.* **2000**, *105*, 1589-1594.
- [134] Tsujii, M.; Kawano, S.; Tsuji, S.; Sawaoka, H.; Hori, M.; Dubois R. N. Cyclooxygenase Regulates Angiogenesis Induced By Colon Cancer Cells. *Cell* **1998**, *93*, 705-716.
- [135] Langman, M. J.; Jensen, D. M.; Watson, D. J.; Harper, S. E.; Zhao, P. L.; Quan, H.; Bolognese, J. A.; Simon, T. J. Adverse Upper Gastrointestinal Effects of Rofecoxib Compared With Nsaids. *J. Am. Med. Assoc.* **1999**, *282*, 1929-1933.
- [136] Williams, J. L.; Borgo, S.; Hasan, I.; Castillo, E.; Traganos, F.; Rigas, B. Nitric-Oxide Releasing Nsaids Alter the Kinetics of Human Colon Cancer Cell Lines More Effectively Than Traditional Nsaids: Implications for Colon Cancer Prevention. *Cancer Res.* **2001**, *61*, 3285-3289.
- [137] Rao, C. V.; Rivenson, A.; Simi, B.; Reddy, B. S. Chemoprevention of Colon Carcinogenesis By Dietary Curcumin, A Naturally Occurring Plant Phenolic Compound. *Cancer Res.* **1995**, *55*, 259-266.
- [138] Rao, C. V.; Desai, D.; Rivenson, A.; Simi, B.; Amin, S.; Reddy, B. S. Chemoprevention of Colon Carcinogenesis By Phenylethyl-3-Methylcaffeate. *Cancer Res.* **1995**, *55*, 2310-2315.
- [139] Kawamori, T.; Lubet, R.; Steele, V. E.; Kelloff, G. J.; Kaskey, R. B.; Rao, C. V.; Reddy, B. S. Chemopreventive Effect of Curcumin, A Naturally-Occurring Antiinflammatory Agent, During the Promotion/Progression Stages of Colon Cancer. *Cancer Res.* **1999**, *59*: 597-601.
- [140] Rao, C. V.; Simi, B.; Reddy, B. S. Inhibition By Dietary Curcumin of Azoxymethane-Induced Ornithine Decarboxylase, Tyrosine Protein Kinase, Arachidonic Acid Metabolism and Aberrant Crypt Foci in the Rat Colon. *Carcinogenesis* **1993**, *14*, 2219-2225.
- [141] Rao, C. V.; Desai, D.; Simi, B.; Kulkarni, N.; Amin, S.; Reddy, B. S. Inhibitory Effects of Caffeic Acid Esters on Azoxymethane-Induced Biochemical Changes and Aberrant Crypt Foci Formation in Rat Colon. *Cancer Res.* **1993**, *53*, 4182-4188.
- [142] Huang, M. T.; Ma, W.; Yen, P.; Xie, J. G.; Han, J.; Frenkel, K.; Grunberger, D.; Conney, A. H. Inhibitory Effects of Caffeic Acid Phenethyl Ester (CAPE) on 12-O-Tetradecanoylphorbol-13-Acetate-Induced Tumor Promotion in Mouse Skin and the Synthesis of Dna, Rna and Protein in Hela Cells. *Carcinogenesis* **1996**, *17*, 761-765.
- [143] Mahmoud, N. N.; Carothers, A. M.; Grunberger, D.; Bilinski, R. T.; Churchill, M. R.; Martucci, C.; Newmark, H. L.; Bertagnolli, M. M. Plant Phenolics Decrease Intestinal Tumors in an Animal Model of Familial Adenomatous Polyposis. *Carcinogenesis* **2000**, *21*, 921-927.
- [144] Xu, Y. X.; Pindolia, K. R.; Janakiraman, N.; Chapman, N.; Gautam, S. C. Curcumin Inhibits Il-1 α and TNF- α Induction of AP-1 and NF- κ B Dna-Binding Activity in Bone Marrow Stromal Cells. *Hematopathol. Mol. Hematol.* **1998**, *11*: 49-62.
- [145] Taylor, J. D. Lipoxygenase Regulation of Membrane Expression of Tumor Cell Glycoproteins and Subsequent Metastasis. *Adv. Prostaglandin Thromboxane Leukot. Res.* **1989**, *19*, 439-443
- [146] Natarajan, K.; Singh, S.; Burke, T. R. Jr; Grunberger, D.; Aggarwal, B. B. Caffeic Acid Phenethyl Ester is a Potent and Specific Inhibitor of Activation of Nuclear Transcription Factor Nf-Kappa B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9090-9095.
- [147] Torrance, C. J.; Jackson, P. E.; Montgomery, E.; Kinzler, K. W.; Vogelstein, B.; Wissner, A.; Nunes, M.; Frost, P.; Discifani, C.M. Ombinatrial Chemoprevention of Intestinal Neoplasia. *Nat. Med.* **2000**, *6*, 1024-1028.
- [148] Gupta, R. A.; Dubois, R. N. Combinations for Cancer Prevention. *Nat. Med.* **2000**, *6*, 974-5.
- [149] Agarwal, B.; Rao, C. V.; Bhendwal, S.; Ramey, W. R.; Shirin, H.; Reddy, B. S. Lovastatin Augments Sulindac-Induced Apoptosis in Colon Cancer Cells and Potentiates Chemopreventive Effect of Sulindac. *Gastroenterology* **1999**, *117*, 838-847.
- [150] Malisetty, V. S.; Cooma, I.; Reddy, B. S.; Rao, C. V. Lamin B, Caspase-3-Activity, and Apoptosis Induction By A Combination of Hmg-Coa Reductase Inhibitor and Cox-2 Inhibitors: A Novel Approach in Developing Effective Chemopreventive Regimens. *Int. J. Oncol.* **2002**, *20*, 753-759.
- [151] Li, H.; Schut, H. A.; Conran, P.; Kramer, P. M.; Lubet, R. A.; Steele V. E. Prevention by Aspirin and Its Combination with α -Difluoromethylornithine of Azoxymethane-Induced Tumors, Aberrant Crypt Foci and Prostaglandin E₂ Levels in Rat Colon. *Carcinogenesis* **1999**, *20*, 425-430.
- [152] Rao, C. V.; Hirose, Y.; Cooma, I.; Reddy, B. S. Modulation of Experimental Colon Tumorigenesis By Types and Amounts of Dietary Fatty Acids. *Cancer Res.* **2001**, *61*, 1927-1933.
- [153] Reddy, B. S.; Rao, C. V. Colon Cancer: A Role for Cyclooxygenase-2 Specific Nonsteroidal Anti-Inflammatory Drugs. *Drugs Aging* **2000**, *6*, 329-334.
- [154] Whelan, J.; Mcentee, M. F. Nonsteroidal Antiinflammatory Drugs, Prostaglandins, and Apc Driven Intestinal Tumorigenesis. In *Cancer Drug Discovery and Development - COX-2 Blockade in Cancer Prevention and Therapy*; Harris, R. E.; Ed, Humana, Press, New Jersey, **2003**, pp.117-145.