

Chemoprevention of Arylamine-Induced Colorectal Aberrant Crypts

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Since recombinant human cyclooxygenase (COX) enzymes have been shown to activate environmental and dietary carcinogens implicated in human colorectal cancer etiology, we hypothesized that COX-2 inhibitors reduce arylamine-induced aberrant crypts (AC) and foci (ACF), preneoplastic lesions of colorectal cancer. Male weanling F344 inbred rats were fed modified AIN-76A control diet or the same diets supplemented with 320 ppm sulindac or 500, 1000, or 1500 ppm celecoxib. At 7 weeks of age, rats received a subcutaneous injection of 3,2'-dimethyl-4-aminobiphenyl (DMABP), an arylamine colon carcinogen, once weekly for two weeks. Ten weeks after the initial DMABP or vehicle treatment (at 17 weeks of age), rats were euthanized with CO₂, and the entire colorectum was removed and scored for ACF and AC. ACF possessing one to five AC were identified in the colorectum of rats administered DMABP, whereas no AC/ACF were identified in vehicle-treated controls. Significant reductions ($p < 0.001$) in ACF and AC frequencies were observed in DMABP-treated rats supplemented with sulindac or celecoxib. Celecoxib reduced AC and ACF more than sulindac, but this difference was not significant ($p > 0.05$). Reductions in both AC and ACF were highest following treatment with 1000 ppm celecoxib. These results provide additional experimental support for the chemopreventive effects of COX inhibitors in arylamine-induced colorectal cancer. *Exp Biol Med* 233:71–75, 2008

Key words: aberrant crypts; sulindac; celecoxib; 3,2'-dimethyl-4-aminobiphenyl; chemoprevention; colorectal cancer

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Introduction

Colorectal cancer is one of the most common preventable malignancies in the Western world (1). Risk factors include dietary ingestion of well-done meats (2) and cigarette smoking (3, 4), resulting in exposures to arylamine carcinogens. Arylamines such as 3,2'-dimethyl-4-aminobiphenyl (DMABP) (5) and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (6) induce colon tumors in the rat.

Aberrant crypts (AC) and aberrant crypt foci (ACF) are specific, dose-dependent preneoplastic lesions that form in response to colorectal carcinogens in rodents (7, 8). ACF also are observed in human colon (9) associated with dysplasia (10) and carcinomas (11). The induction of colorectal AC/ACF is particularly useful for chemoprevention studies because they provide quantifiable preneoplastic markers that correlate very highly with colon tumors (12).

The chemopreventive effects of non-steroidal anti-inflammatory drugs (NSAIDs) have been demonstrated in population-based epidemiology studies and in clinical trials (13). NSAIDs such as sulindac are nonselective inhibitors of cyclooxygenase-1 (COX-1) and 2 (COX-2), whereas NSAIDs such as celecoxib are selective inhibitors of COX-2. COX-1 is constitutively expressed in many tissues, whereas COX-2 is negligible in most tissues (14). However, COX-2 is over-expressed in human colorectal carcinomas, and higher expression of COX-2 correlates with poor prognosis (15).

Preclinical models have been very useful to evaluate the utility of chemopreventive agents for the prevention of colorectal cancer (16). Since spontaneous epithelial tumors of the colon are rare in experimental animals, colon tumors have frequently been generated by administration of chemical carcinogens. Although previous studies have reported that sulindac and celecoxib reduce azoxymethane-induced colorectal carcinogenesis in rats (17), no studies to our knowledge have investigated the effects of these agents on arylamine-induced colorectal cancer. Thus, we undertook the present study to test the effects of sulindac and celecoxib on DMABP-induced AC/ACF in the rat.

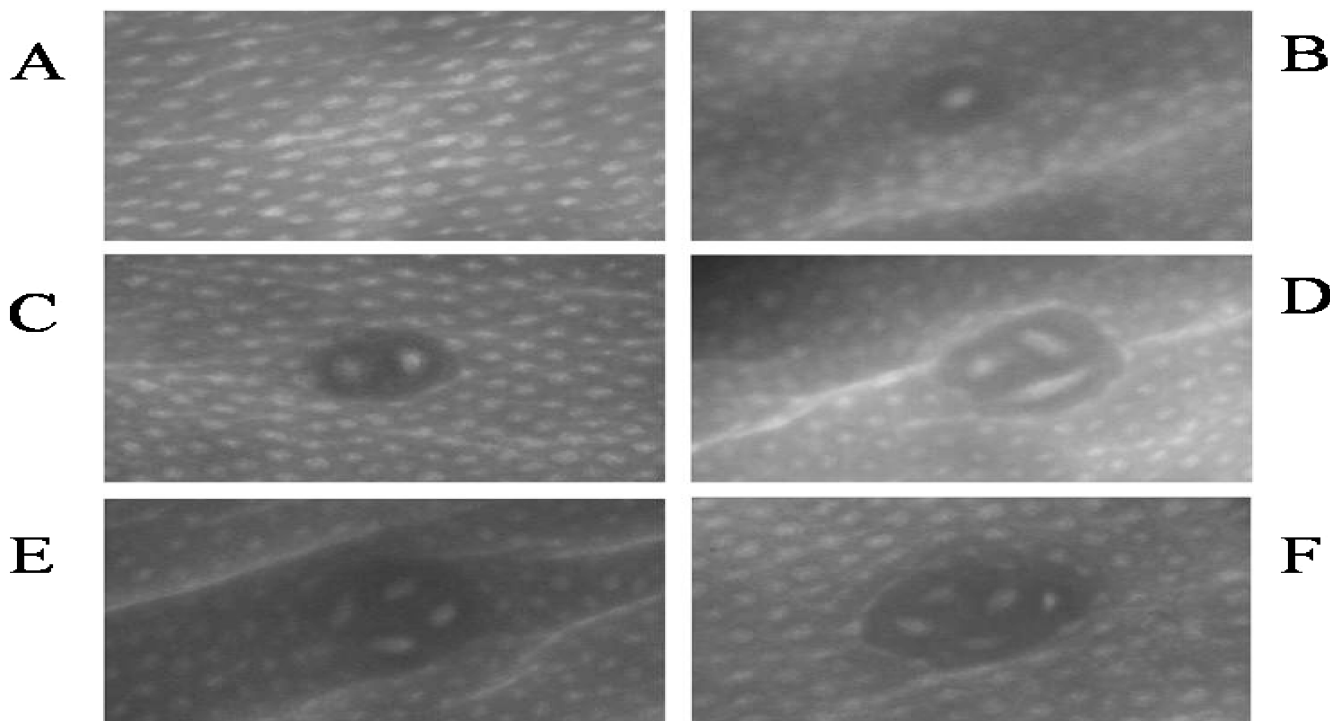


Figure 1. Identification of ACF in rats fed control diet and treated with DMABP. A, normal mucosa; B, single aberrant crypt; C, two aberrant crypts/focus; D, three aberrant crypts/focus; E, four aberrant crypts/focus; F, five aberrant crypts/focus.

Materials and Methods

Chemicals. DMABP was purchased from Toronto Research Chemicals, Inc. (Toronto, Canada). Sulindac, peanut oil, and sodium chloride were obtained from Sigma Chemical Co. (St. Louis, MO). Methylene blue was obtained from Eastman Kodak Company (Rochester, NY). Celecoxib was kindly provided by Pfizer Inc. (Groton, CT).

Animals and Diets. Male weanling F344 inbred rats were purchased from Charles River Laboratories (Wilmington, MA) and were housed 2–3 per cage in a temperature-controlled room with 12-hour cycles of light and dark in the animal facilities of the University of Louisville School of Medicine. The sulindac and celecoxib feeding protocol and dose levels were selected based on previous studies of azoxymethane-induced ACF (16). All rats were provided tap water and modified AIN-76A (casein, 20%; DL-methionine, 0.3%; corn starch, 52%; dextrin, 13%; corn oil, 5%; cellulose, 5%; mineral mix-AIN-76, 3.5%; vitamin mix-AIN-76, 1%; and choline bitartrate, 0.2%) control diet (Bio-Serv, Frenchtown, NJ) for one week prior to the study. Rats (five weeks of age) were then separated randomly into 5 groups and fed the modified AIN-76A diet (control) or the modified AIN-76A diet supplemented with 320 ppm sulindac or 500, 1000, or 1500 ppm celecoxib for the length of the study.

Treatment. Two weeks after dietary treatment (at seven weeks of age), all rats received a subcutaneous injection of DMABP (100 mg/kg in peanut oil) or peanut oil

vehicle once weekly for two weeks as described previously (18).

Identification and Quantitation of AC and ACF. Ten weeks after the initial DMABP or vehicle treatment (at 17 weeks of age), rats were euthanized with CO₂ and the entire colorectum was removed, flushed with 0.9% sodium chloride, opened longitudinally, and fixed flat between paper towels in 70% ethanol. The fixed colorectum were stained for 1 min with 0.1% methylene blue in 0.9% sodium chloride. ACF were identified under a dissecting microscope. The number of ACF and AC were scored blind as previously described (18).

Statistical Analysis. Differences in response between groups were tested for significance by one-way analysis of variance followed by Bonferroni's correction for multiple comparisons.

Results

DMABP-treated rats were fed diets supplemented with 320 ppm sulindac or 500, 1000, or 1500 ppm celecoxib. Sulindac is a nonspecific COX enzyme inhibitor (both COX-1 and COX-2), whereas celecoxib is a selective inhibitor of COX-2. ACF possessing one to five AC were identified in the colorectum of rats administered DMABP (Fig. 1), whereas no AC/ACF were identified in vehicle-treated controls.

ACF and AC frequencies were significantly ($p < 0.001$) reduced in DMABP-treated rats supplemented with 320 ppm sulindac or 500, 1000, or 1500 ppm celecoxib (Fig. 2).

Celecoxib reduced AC/ACF more than sulindac, but this difference was not significant ($p>0.05$). Reductions in both AC and ACF were highest following treatment with 1000 pm celecoxib.

Discussion

The use of NSAIDs in the chemoprevention of colorectal cancer is of particular interest. As recently reviewed, molecular studies as well as investigations in humans and animal models support the hypothesis that aberrant induction of COX-2 and upregulation of the prostaglandin cascade play a significant role in carcinogenesis, and that blockage of these processes is a strategy for cancer prevention (19). Prostaglandin H Synthase catalyzes the metabolism of arachidonic acid in prostanoic acid biosynthesis. Two distinct isoforms of this enzyme, COX-1 and COX-2, have been identified. COX-1 is expressed in most extrahepatic tissues, whereas COX-2 is present at lower levels under physiological conditions but its expression is induced by a variety of stimuli (14), including polycyclic aromatic and heterocyclic amines (20).

COX-2 plays a significant role in tumor growth (21) and angiogenesis (22). Williams and colleagues transplanted tumor grafts from the Lewis lung carcinoma cell line to COX-2 null mice and COX-2 wild-type mice. The growth of tumor grafts was slower in COX-2 null mice, whereas COX-1 did not play a significant role in tumor growth in this animal model (22). Oshima and co-workers demonstrated that COX-2 inhibition significantly reduced tumor multiplicity of intestinal polyps in mice with a mutant adenomatous polyposis coli allele (23). In addition to stimulation of the tumor growth and angiogenesis, COX-2 also protects the cells from apoptosis. Several studies have shown that COX-2 inhibitors increase apoptosis in colorectal carcinoma cell lines via the activation of different signal transduction pathways (24–26).

Peroxidative activation of carcinogenic aromatic amines via prostaglandin H synthase has been reported (27, 28). Recombinant human COX-1 and COX-2 enzymes have been shown to activate aromatic and heterocyclic amine carcinogens to intracellular electrophiles that bind covalently to DNA (29). Several studies in animal models support a role for COX in chemically-induced carcinogenesis. For example, acetaminophen treatment substantially reduced DMABP-induced colonic tumors (30) and numerous other parameters of neoplastic development of colon cancer (31). Docosahexanoic acid treatment reduced formation and growth of 2-amino-1-methyl-6-phenylimidazo(4,5-b) pyridine-induced ACF in rat colon (32). Recently, celecoxib was shown to reduce DNA adduct levels in colon of rats treated with DMABP (33). Thus, we hypothesized that sulindac and celecoxib as inhibitors of COX-1 and/or COX-2 would reduce colon AC/ACF resulting from exposure to DMABP. Sulindac and celecoxib reduced ACF and AC in colons of rats treated with the

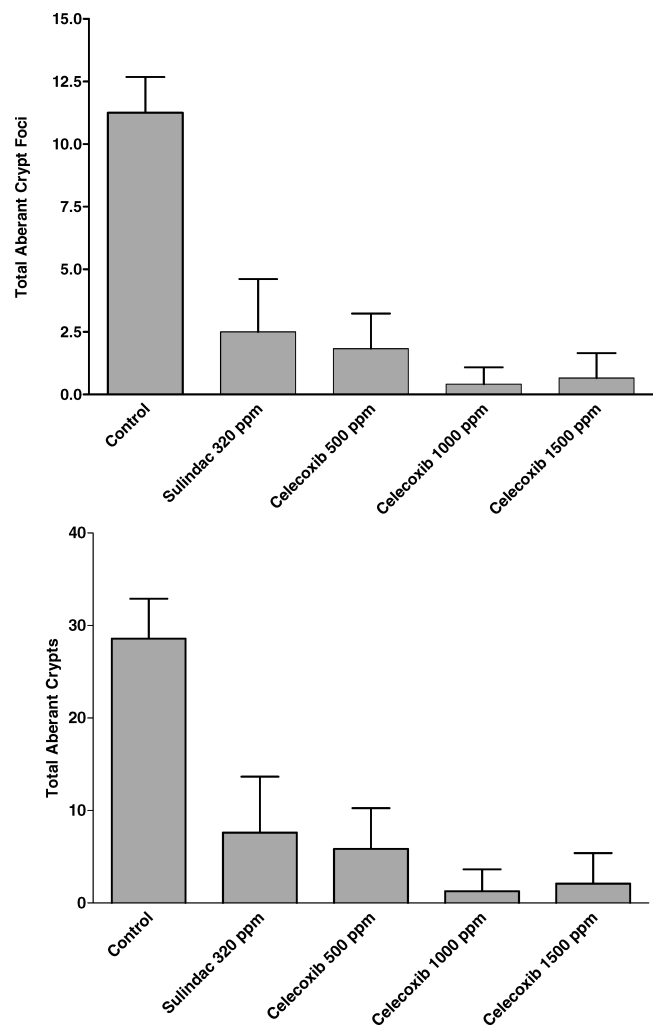


Figure 2. ACF (top panel) and AC (bottom panel) in DMABP-treated animals fed with the diets listed on the abscissae. Each bar represents mean + SE for 12 rats. Both ACF and AC differed significantly ($p<0.001$) between rats fed control for each treatment. Differences between various doses of sulindac and celecoxib were not significant ($p>0.05$).

model arylamine carcinogen DMABP, providing further experimental support for the chemopreventive effects of COX inhibitors in arylamine-induced colorectal cancer.

Long-term use of nonspecific COX-1 and COX-2 inhibitors is associated with serious gastrointestinal side effects that have been attributed to inhibition of COX-1, which mediates production of prostaglandins in the gastric mucosa. Selective COX-2 inhibitors such as celecoxib were developed to avoid these side effects, which have been verified in clinical studies (34). However, celecoxib and other COX-2 inhibitors appear to increase the risk of cardiovascular side effects such as hypertension, stroke, and myocardial infarction (35), although other studies such as the Prevention of Spontaneous Adenomatous Polyps Trial did not find celecoxib associated with increased cardiovascular risk (13).

Sulindac was shown to reduce both the frequency and

diameter of colonic polyps in patients with familial adenomatous polyposis (36). Celecoxib treatment in patients with familial adenomatous polyposis resulted in significant reductions in mean number of colorectal polyps as well as in overall polyp burden (37). Furthermore, celecoxib significantly reduced the occurrence of colorectal adenomas in the Prevention of Colorectal Sporadic Adenomatous Polyps trial, a randomized, placebo-controlled, double-blind study of celecoxib given daily in a single 400-mg dose at 107 centers in 32 countries (38). The results of the present study showed that both sulindac and celecoxib reduced AC and ACF in rats administered DMABP, providing additional evidence that COX inhibitors reduce risk of human colorectal cancer associated with arylamine exposures.

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