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# Do NSAIDs Exert Their Colon Cancer Chemoprevention Activities Through the Inhibition of Mucosal Prostaglandin Synthetase?

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**Abstract** Nonsteroidal antiinflammatory drugs (NSAIDs) have considerable potential as chemopreventive agents for colorectal cancer. Recent case-control drug surveillance and large cohort studies found that patients with regular aspirin use had a reduced incidence of colorectal cancer and/or decreased death rate from this disease. Several different NSAIDs reduce formation of both colon adenomatous polyps (the precursor lesion of colon cancer) and cancers in experimental animals given known carcinogens. Perhaps most convincing are reports that the NSAID sulindac promotes regression and inhibits recurrence of adenomatous colon polyps in patients with adenomatous polyposis coli. The best characterized pharmacologic effect of the NSAIDs is their reduction of prostaglandin synthesis by inhibiting prostaglandin synthetase $PGE_2$, which catalyzes the formation of prostaglandin precursors from arachidonic acid. Several lines of evidence are contrary to the concept that inhibition of prostaglandin synthesis is central to the NSAIDs' chemopreventive effects. Relatively high levels of prostaglandins have been reported to inhibit tumor cell growth both *in vivo* and *in vitro*, and to inhibit differentiation in some tumor cell lines. We evaluated comparative chemopreventive effects on colon tumor formation in an azoxymethane (AOM)-induced colon carcinogenesis rat model using the NSAIDs piroxicam, sulindac, and sulindac sulfone, a metabolite of sulindac which lacks the anti-prostaglandin synthetase activity typically associated with NSAID-induced gastrointestinal toxicities. The results demonstrate that sulindac sulfone, a compound lacking anti-prostaglandin synthetase activity, inhibits AOM-induced colon cancer in rats. Substantial dose-dependent reductions in both tumor burden and tumor multiplicity were observed in the sulindac sulfone-treated animals. Although both piroxicam and sulindac significantly reduced rat colonic mucosal $PGE_2$ levels to less than 50% of their AOM control value, even the highest dietary concentration of sulindac sulfone had no statistically significant effect on mucosal $PGE_2$ concentrations. These results suggest that NSAIDs do not exert their colon cancer chemoprevention activities through the inhibition of mucosal prostaglandin synthetase. 

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**Key words:** Colon cancer prevention, NSAIDs, sulindac, sulindac sulfone

According to recent National Cancer Institute data, colorectal cancer remains the second leading cause of cancer death in the United States. Despite improvements in surgical techniques and the development of active adjuvant chemotherapy for advanced disease, five-year cure rates remain low. The most promising strategies to reduce colorectal cancer mortality involve developing improved screening methods and novel chemopreventive approaches.

Non-steroidal antiinflammatory drugs (NSAIDs) are the most promising chemopreventive agents for colorectal cancer. Three case-control drug surveillance studies and one large cohort study found that patients with regular aspirin use had a reduced incidence or decreased death rate from colorectal cancer [1–4]. Furthermore, recently published case-control [5] and cohort [6] studies show a significantly lower incidence of colorectal adenomas in subjects reporting regular aspirin intake. Among patients enrolled in a randomized trial of antioxidant vitamins, those who took aspirin regularly had an appreciably lower probability of having an adenoma detected at their one year follow-up colonoscopy [7]; however, in the Physicians' Health Study [8], regular aspirin use, at a dose adequate for preventing myocardial infarction, was not associated with a substantial reduction in the incidence of either colorectal adenomas or cancer during five years of randomized treatment follow-up.

A variety of different NSAIDs have reduced the formation of both colon adenomatous polyps and cancers in experimental animals administered known carcinogens [9–15]. Also, NSAIDs inhibited the growth and clinical expression of transplanted tumors and metastatic cancer spread in animal models [16–20], and potentiated the antitumor effects of immunotherapy, radiotherapy and anticancer drug treatment [21–26].

Perhaps the most convincing data concerning the potential role of NSAIDs as chemopreventive agents for colorectal adenomas and ultimately cancer comes from reports that sulindac promotes regression and inhibits recurrence of adenomatous colon polyps in patients with familial adenomatous polyposis (FAP) [27–31]. Of especial interest, a randomized, double-blind, placebo-controlled study of 22 patients with FAP performed by Giardiello *et al.* [30] showed that sulindac treatment (150 mg orally twice daily for 9 months) was associated with a statistically significant decrease in the mean number of polyps and their mean diameter. When sulindac treatment was stopped at 9 months, the number of polyps had decreased to 40% of baseline values and the diameter of the polyps to 35% of baseline values ($p=.01$, $p<.001$, respectively, for comparison with changes observed in the placebo group). Furthermore, three months after treatment with sulindac was terminated, both the number and the size of the adenomas increased in sulindac-treated patients.

NSAIDs were first suggested as possible therapy for FAP because they were known to inhibit intracellular prostaglandin synthetase, and some colonic tumors had been observed to produce excessive quantities of prostaglandins [32,33]. Narisawa *et al.* [34] reported a high level of $PGE_2$ in local venous blood draining colon carcinomas and in peripheral blood of patients with liver or lung metastases. Tissue analysis showed a significantly larger amount of $PGE_2$ production in carcinoma versus normal colonic mucosal tissue. These investigators suggested that increased local blood $PGE_2$ could enhance metastasis. We have performed a rat colon carcinogenesis study to evaluate whether an NSAID-like compound lacking antiprostaglandin synthetase activity (*e.g.*, sulindac sulfone) is capable of inhibiting colon tumor growth.

## MATERIALS AND METHODS

### Animals, Diets and Carcinogen

Male Fisher-344 rats (Harlan Laboratories) were fed AIN-76A purified rodent diets (Dyets, Inc., Bethlehem, PA) prior to randomization. Rats were housed three to a cage at 72°F on a 12-hour each day/night cycle. Two hundred ten (210) rats were randomized to seven groups with azoxymethane (AOM, Sigma Chemicals, St. Louis, MO) administered subcutaneously (15 mg/g) weekly for two consecutive weeks

(total, 30 mg/kg). Piroxicam, sulindac and sulindac sulfone were administered in the daily diet two weeks after first dose of AOM. Rats were sacrificed by $CO_2$ euthanasia at 31 weeks. At autopsy, the entire gastrointestinal tract from the stomach to the anus was removed, cleaned, and fixed in 10% formalin. Any mass found in the colon was removed, embedded in paraffin, and processed by routine histological procedures. The sections were stained with hematoxylin and eosin and examined histologically to determine the tumor type. Neoplasms were classified as adenomas if there was no evidence of invasion through the muscularis mucosa. Adenocarcinomas ranged from well- to poorly differentiated malignant tumors invading across the muscularis mucosa and were frequently mucinous.

$PGE_2$ concentrations were assayed by radioimmunoassay (RIA) in fresh, unfixed colonic mucosa which was placed immediately in indomethacin and stored in liquid nitrogen after punch biopsies were obtained from the distal colon.

### Extraction of $PGE_2$ from Colonic Mucosal Biopsies

The frozen biopsies were placed into 1.0 ml of 0.05 M Tris-HCl buffer, pH 7.4, containing 5 mg indomethacin/ml which instantaneously and reliably halts *in vitro* $PGE_2$ synthesis. Tissue was homogenized in a siliconized 5.0 ml glass tissue grinder for 30 seconds, and 100 µl were removed for protein determinations. One hundred percent ethanol (EtOH) (2.0 ml) was added to the remaining homogenate and allowed to stand for 5 minutes on ice. Distilled water (10.3 ml) was added to the homogenate (final concentration, 15% EtOH) and the sample was centrifuged 10 minutes at 400 x g at 4°C. The supernatant was removed and the pH was adjusted to 3.0 with 0.25 M HCl. The sample was then applied to a C18 silica column previously washed with 20 ml 100% EtOH followed by 20 ml distilled water. The column was rinsed with 20 ml of a 15% EtOH solution followed by 20 ml petroleum ether. $PGE_2$ was gravity-eluted with 10 ml methyl formate. The methyl formate was divided into 4 equal 2.5 ml aliquots, dried under nitrogen, and stored at –80°C. Samples were reconstituted in 0.25 ml assay buffer and assayed for $PGE_2$ content using a Dupont $^{125}$I-$PGE_2$ RIA kit.

### Statistical Methods

The significance of differences between the AOM control group and drug-treated groups with respect to tumor incidence was determined using Poisson regression analysis. To test the significance of differences between AOM control and drug-treated groups with respect to tumor burden (the sum of sizes of tumors per rat), an Analysis of Covariance Model was used; p-values $<.05$ were considered statistically significant. Means are reported ± standard error (SE).

## RESULTS

### NSAID Suppression of Colon Tumors

AOM-control animals had an average of 2.0 ± 3.1 colon tumors per animal at study termination. Piroxicam in a dietary dose of 0.015% reduced the number of tumors per rat colon to 38.5% of the AOM control. Likewise, sulindac in a dietary dose of 0.04% reduced the number of tumors per rat colon to 26.5% of the AOM control.

Sulindac sulfone treatment resulted in a dose-dependent reduction of both the total tumor burden (sum of tumor sizes) and the mean number of tumors per rat colon. A statistically significant decrease in the number of colon tumors per animal was observed in animals treated with 0.20% and 0.10% sulindac sulfone. The number of tumors per animal in the 0.20% sulfone-treated group was reduced to 21.5% of the AOM control. Animals treated with the 0.10% dietary dose of sulindac sulfone also experienced a statistically significant reduction in tumors (per rat) to 45.0% of the AOM control.

### Inhibitory Effect of NSAIDs on $PGE_2$ Content in Colonic Mucosa

The colonic mucosa of AOM control animals contained 3.9 ± 0.5 ng $PGE_2$/mg protein. Piroxicam (0.015%) significantly reduced the mean $PGE_2$ content to 17.3% of the AOM control. Likewise, sulindac (0.04%) reduced the $PGE_2$ levels to 40.3% of the AOM control value. In contrast, neither of the two highest dietary concentrations of sulindac sulfone was associated with significant reductions in $PGE_2$ content per rat colon. For example, the 0.20% dietary dose of sulindac

sulfone was associated with a non-significant reduction of $PGE_2$ content to 78.6% of the AOM control ($p>.01$). Sulindac sulfone in the lower dietary concentration (0.10%) was associated with a mean $PGE_2$ content that was 105.4% of the AOM control.

## DISCUSSION

The mechanism by which sulindac sulfone inhibits colon tumorigenesis is unknown, but does not appear to be mediated only by anti-prostaglandin synthetase activity. The best characterized pharmacologic effect of the NSAIDs is to diminish prostaglandin synthesis by inhibiting prostaglandin synthetase, which catalyzes the formation of prostaglandin precursors from arachidonic acid. Prostaglandins are potent mediators of numerous biological responses and probably play a role in maintaining cellular viability and modulating both normal and neoplastic cell proliferation [32,35,36]. A variety of experimental animal and human tumors contain and/or synthesize large amounts of prostaglandins [32,33].

Other lines of evidence are contrary to the concept that inhibition of prostaglandin synthesis is central to the NSAID colon cancer chemopreventive effect. Relatively high levels of prostaglandins have been reported to inhibit tumor cell growth both *in vivo* and *in vitro* and to inhibit differentiation in some tumor cell lines [32,36–38]. Exogenous prostaglandins have inhibited basal mucosal DNA synthesis in colon explants from animals [39]. DeMello *et al.* [40] reported that NSAID concentrations which inhibit cell growth in rat hepatoma and human fibroblast cell lines *in vitro* correlate poorly with concentrations reported to inhibit cyclooxygenase in other studies. In addition, prostaglandin synthetase activity was not detected in the rat hepatoma cell line, and exogenous prostaglandins did not reverse the antiproliferative effects of indomethacin.

The AOM colon carcinogenesis model has been useful for studying the inhibition of colonic neoplasia by a variety of NSAIDs, including sulindac and piroxicam. In the current study, sulindac and piroxicam were included as positive controls at doses shown to be efficacious in other studies. Our results with sulindac sulfone are consistent with the concept of DeMello *et al.* [40]; tumor growth inhibition by NSAIDs may not depend on their anti-prostaglandin synthetase activity. Sulindac sulfone, reported to lack prostaglandin inhibitory activity [41,42] is a metabolite resulting from the irreversible oxidation of the parent compound, sulindac [41].

Mechanisms postulated to explain the antiproliferative/antitumor effects of NSAIDs other than prostaglandin modulation include the following: interference with a spectrum of membrane-associated processes, including G protein function, transmembrane calcium flux, and cell-to-cell binding [43,44]; inhibition of activity of other enzymes (in addition to cyclooxygenase), including phosphodiesterase and cyclic AMP-dependent protein kinase which may be integral to cancer initiation and promotion [45]; inhibition of cylooxygenase co-oxidation of non-lipid substrates to carcinogenic derivatives during prostaglandin synthesis [46,47]; enhancement of a multitude of immunological responses which may have an important role in restoring host antitumor immunity [48]; and finally mechanisms related to the induction of cellular apoptosis.

Of course, the ultimate role of NSAIDs in the chemoprevention of colon cancer will depend on the outcome of well-controlled, Phase III clinical trials. Although sulindac has proven an effective inhibitor of colorectal polyp growth in FAP patients, the majority often experience severe upper gastrointestinal side effects with longterm use related to its inhibition of prostaglandin synthetase. The sulfone metabolite of sulindac appears to lack this enzyme inhibitory activity and may prove an equally effective, but better tolerated compound for chronic use in FAP patients and those individuals with a history of sporadic colorectal adenomas.

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## REFERENCES

1. Rosenberg L, Palmer J, Zanber AG, Warshauer ME, Stolley PD, Shapiro S: A hypothesis: Nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. J Natl Cancer Inst 83:355–358,

1991.
2. Kune G, Kune S, Watson LF: Colorectal cancer risk, chronic illnesses, operations, and medications: Case control results from the Melbourne Colorectal Cancer Study. Cancer Res 48:4399–4404, 1988.
3. Pelage II, Maibach HT, Brown SH, Wilcox CM: Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. Arch Intern Med 154:394–399, 1994.
4. Thun M, Namboodiri M, Heath CW: Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 325:1593–1596, 1991.
5. Logan RFA, Little J, Hawtin PG, Hardcastle JD: 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 307:285–289, 1993.
6. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC: Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med 121:241–246, 1994.
7. Greenberg ER, Baron JA, Freeman DH Jr, Mandel JS, Haile MR: Reduced risk of large-bowel adenomas among aspirin users. J Natl Cancer Inst 85:912–916, 1993.
8. Gann PH, Manson JE, Glynn RJ, Buring JE, Hennekens CH: Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst 85:1220–1224, 1993.
9. Tanaka T, Kojima T, Yoshimi N, Sugie S, Mori H: Inhibitory effect of the non-steroidal anti-inflammatory drug, indomethacin on the naturally occurring carcinogen, 1-hydroxyanthraquinone in male ACI/N rats. Carcinogenesis 12:1949–1952, 1991.
10. Pollard M, Luckert P: Prevention and treatment of primary intestinal tumors in rats by piroxicam. Cancer Res 49:6471–6475, 1989.
11. Reddy B, 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 47:5340–5346, 1987.
12. Reddy BS, Tokumo K, Kulkarni N, Aligia C, Kelloff G: Inhibition of colon carcinogenesis by prostaglandin synthesis inhibitors and related compounds. Carcinogenesis 13:1019–1023, 1992.
13. Narisawa T, Hermanek P, Habs M, Schmahl D: Reduction of carcinogenecity of *N*-nitrosomethylurea by indomethacin and failure of resuming effect of prostaglandin $E_2$ ($PGE_2$) against indomethacin. J Cancer Res Clin Oncol 108:239–242, 1984.
14. Skinner S, Penney A, Penney G, O'Brien PE: Sulindac inhibits the rate of growth and appearance of colon tumors in the rat. Arch Surg 26:1094–1096, 1991.
15. Reddy BS, Rao CV, Riverson A: Aspirin inhibits cancer of the colon. Gastroenterology 104:A443, 1993.
16. Hail V, Horakova Z, Shaff RE, Beaven MA: Alteration of tumor growth by aspirin and indomethacin: Studies with two transplantable tumors in mouse. Eur J Pharmacol 37:367–376, 1976.
17. Tanaka Y, Tanaka T, Ishitsuka H: Antitumor activity of indomethacin in mice bearing advanced colon 26 carcinoma compared with those with early transplants. Cancer Res 49:5935–5939, 1989.
18. Gasic G, Gasic T, Galanti N, Johnson T, Murphy S: Platelet-tumor interactions in mice. The role of platelets in the spread of malignant disease. Int J Cancer 11:704–718, 1973.
19. Honn K, Busse W, Sloane BF: Prostacyclin and thromboxanes. Implications for their role in tumor cell metastasis. Biochem Pharmacol 32:1–11, 1983.
20. Costantini V, Fuschiotti P, Allegrucci M, Agnelli G, Nenci GG, Fioretti MC: Platelet-tumor cell interaction: Effect of prostacyclin and a synthetic analog on metastasis formation. Cancer Chemother Pharmacol 22:289–293, 1988.
21. Bennett A: Effect of prostaglandin synthesis inhibitors on tumor growth *in vivo*. In Powles TJ (ed): "Prostaglandins and Cancer. First International Conference." New York: Liss, 1982, pp 759–766.
22. Lynch N, Salomon J: Tumor growth inhibition and potentiation of immunotherapy by indomethacin in mice. J Natl Cancer Inst 62:117–212, 1979.
23. Bennett A, Houghton J, Leaper KJ, Stamford IF: Cancer growth, response to treatment and survival time in mice: Beneficial effect of the prostaglandin synthesis inhibitor flurbiprofen. Prostaglandins 17:179–191, 1979.
24. Bennett A, Berstock A, Carroll MA: Increased survival of cancer-bearing mice treated with inhibitors of prostaglandin synthesis alone or with chemotherapy. Br J Cancer 45:762–768, 1982.
25. Bennett A, Gaffen J, Chambers E: Cyclo-oxygenase inhibitors and cell killing by cytotoxic drugs. Adv Prostaglandin Thromboxane Leukot Res 19:427–430, 1989.
26. Milas L, Furuta Y, Hunter N, Nishiguchi I, Runkel S: Dependence of indomethacin-induced potentiation of murine tumor radioresponse on tumor host immunocompetence. Cancer Res 50:4473–4477, 1990.
27. Waddell W, Gasner G, Cerise EJ, Loughry RW: Sulindac for polyposis of the colon. Am J Surg 124:83–87, 1989.
28. Labayle D, Fischer D, Vielh P, Drouhin F, Pariente A, Bories C, Duhamel O, Trousset M, Attali P: Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology 101:635–639, 1991.
29. Rigau J, Pique J, Rubio E, Planas R, Tarrech JM, Bordas JM: Effects of long-term sulindac therapy on colonic polyposis. Ann Int Med 115:952–954, 1991.
30. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, Booker SV, Robinson CR, Offerhaus JA: Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 328:1313–1316, 1993.
31. Winde G, Gumbinger HG, Osswald H, Kemper F, Bunte H: The NSAID sulindac reverses rectal adenomas in colectomized patients with familial adeno-

matous polyposis: Clinical results of a dose-finding study on rectal sulindac administration. Int J Colorectal Dis 8:13–17, 1993.
32. Karmali R: Prostaglandins and cancer. CA: A Cancer J Clin 33:322–332, 1983.
33. Earnest D, Hixson L, Fennerty MB, Emerson SS, Alberts DS: Inhibition of prostaglandin synthesis: Potential for chemoprevention of human colon cancer. Cancer Bull 43:562–568, 1991.
34. Narisawa, T, Kusaki H, Yamasaki Y: Relationship between blood plasma prostaglandin $E_2$ and liver and lung metastases in colorectal cancer. Dis Rectum 33:840–845, 1990.
35. Lupulescu A: Enhancement of carcinogenesis by prostaglandins. Nature 272:634–636, 1978.
36. Tutton J, Barkla D: Influence of prostaglandin analogues on epithelial cell proliferation and xenograft growth. Br J Cancer 41:47–51, 1980.
37. Goodlad R, Mandir N, Levin S, Allen JL, Wright NA: Prostaglandins and the colonic epithelium. Gastroenterology 101:1229–1234, 1991.
38. Santoro M, Philpott G, Jaffe BM: Inhibition of tumor growth *in vivo* and *in vitro* by prostaglandin. Nature 263:777–779, 1976.
39. Craven P, Saito R, DeRubertis R: Role of local prostaglandin synthesis in the modulation of proliferative activity of rat colonic epithelium. J Clin Invest 72:1365–1375, 1983.
40. DeMello M, Bayer B, Beaven MA: Evidence that prostaglandins do not have a role in the cytostatic action of anti-inflammatory drugs. Biochem Pharmacol 29:311–318, 1980.
41. Duggan DE, Hare LE, Ditzler CA, Leib W, Kwan KC: The disposition of sulindac. Clin Pharmacol Ther 21:326–335, 1977.
42. Shen TY, Ham EA, Cirillo VJ, Zanetti M: Structure-activity relationship of certain prostaglandin synthetase inhibitors. In Robinson HJ, Vane JR (eds): "Prostaglandin Synthetase Inhibitors." New York: Raven Press, 1974, pp 18–31.
43. Abramson SB, Lesczynska-Piziak J, Haines K, Reibman J: Non-steroidal anti-inflammatory drugs: Effects on GTP binding protein within the neutrophil plasma membrane. Biochem Pharmacol 41:1567–1573, 1991.
44. Abramson S, Korchak H, Ludewig R, Edelson H, Haines K, Levin RI, Herman R, Rider L, Kimmel S, Weissman G: Modes of action of aspirin-like drugs. Proc Natl Acad Sci 82:7227–7231, 1985.
45. Kantor H, Hampton M: Indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase. Nature 276:841–842, 1978.
46. Craven P, Neidig M, DeRubertis FR: Fatty acid-stimulated oxidation of methylazoxymethanol by rat colonic mucosa. Cancer Res 45:1115–1121, 1985.
47. Wild D, Degen G: Prostaglandin H synthetase-dependent mutagenic activation of heterocyclic aromatic amines of the IQ-type. Carcinogenesis 8:541–545, 1987.
48. Abramson S, Weissmann G: The mechanisms of action of nonsteroidal antiinflammatory drugs. Arthritis Rheum 32:1–9, 1989.