Effect of Aspirin on Prostaglandin E_2 and Leukotriene B_4 Production in Human Colonic Mucosa from Cancer Patients[1]

Thomas O. Frommel,[2] Madhavi Dyavanapalli, Todd Oldham, Nadeem Kazi, Helen Lietz, Youlian Liao, and Sohrab Mobarhan

Division of Digestive Diseases and Nutrition, Department of Medicine [T. O. F., M. D., T. O., N. K., H. L., S. M.] and Department of Preventive Medicine and Epidemiology [Y. L.], Loyola University Medical Center, Maywood, Illinois 60153

ABSTRACT

Results from epidemiological studies indicate that chronic administration of aspirin reduces the incidence of colon cancer. The mechanism that accounts for this reduction is not known, but it may be related to the decreased production of prostanoids that results from aspirin inhibition of cyclooxygenase. However, it is not known whether aspirin has a local effect on prostanoid production in the colonic mucosa and whether this effect is dose dependent. In this study, we determined the effect of oral administration of aspirin on the production of the prostanoid prostaglandin E_2 (PGE_2) in the intact human colonic mucosa. Inhibition of cyclooxygenase could result in an increased availability of arachidonic acid and a corresponding increase in production of other eicosanoids. To determine whether such an effect occurs, we also quantitated the concentration of leukotriene B_4 (LTB_4) in colonic mucosal samples. Mucosal samples were obtained during sigmoidoscopy from the colons of 17 subjects with a history of colonic cancer prior to and following 60 days of self-administration of 325 mg aspirin/day and again 60 days after administration of 650 mg aspirin/day. PGE_2 and LTB_4 concentrations were determined by enzyme immunoassay for tissue samples that were flash frozen after removal from the biopsy forceps and also in medium that was collected from tissue samples that were incubated for 4 h following removal from the subject. PGE_2 concentrations were decreased significantly in samples collected after 60 days of consumption of 325 mg aspirin. An additional 60 days of consuming 650 mg aspirin/day did not result in a further significant decrease relative to that attained after consumption of 325 mg/day. Similar results were obtained using colonic explants, and the addition of aspirin to medium further reduced PGE_2 production. LTB_4 in tissue and medium was not significantly different in pre-*versus* post-aspirin samples, with the exception of an increased concentration in medium samples collected after consumption of 650 mg/day relative to pre-aspirin samples. The results indicate that aspirin affects eicosanoid production in the colonic mucosa of humans, but the effect is most likely restricted to products of the cyclooxygenase-dependent pathway. It appears that 325 mg of aspirin is sufficient to affect PGE_2 production and that increasing the dosage to 650 mg daily provides an additional decrease in PGE_2 synthesis. However, the higher dosage was associated with a considerable increase in complaints of gastric discomfort. Additional study is needed to establish whether doses less than 325 mg also provide a significant decrease in PGE_2 production.

INTRODUCTION

Results from a number of epidemiological studies suggest that aspirin inhibits the initiation and/or progression of colon cancer in humans. In a study of individuals consuming aspirin on a regular basis, the incidence of adenomas in the large bowel was reported decreased by 50% (1). A similar decrease was noted in the incidence of colorectal cancer in those consuming NSAIDs,[3] most of which was aspirin, a minimum of four times each week (2). A slightly greater decrease in colorectal cancer was reported for those consuming two or more aspirin per day, and a decreased incidence of 80% was reported for individuals consuming aspirin regularly for more than 5 years (3, 4). In a large study involving a cohort of more than 650,000 individuals, the risk of colorectal cancer was reduced 40% by consumption of aspirin at least 16 days each month for at least 1 year (5). In contrast, regular aspirin use has been reported by others to reduce the incidence of colorectal cancer by only 10%, and the results of one study indicate that aspirin use increases the risk of colorectal cancer by 50% (6, 7). The results from the recently completed Nurses' Health Study indicate that consuming aspirin a minimum of twice each week decreases the risk of colorectal cancer, but the effect is not realized until 10 or perhaps 20 years following commencement of regular aspirin consumption (8). The data from animal studies are in general agreement with the results from human studies that aspirin has a colon cancer-preventative effect (9, 10). However, in contrast to other NSAIDs, data suggest that aspirin must be administered within a short period following introduction of a carcinogen to limit the

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[2] To whom requests for reprints should be addressed, at Department of Medicine, Building 117, Loyola University Medical Center, 2160 South 1st Avenue, Maywood, IL 60153. Phone: (708) 216-4783; Fax: (708) 216-4113.

[3] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; LTB_4, leukotriene B_4.

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occurrence, growth, and size of subsequent colonic tumors in rodents (9).

The mechanism by which aspirin reduces the incidence of colon cancer is not known. As a specific irreversible inhibitor of cyclooxygenase, aspirin reduces both prostaglandin and thromboxane synthesis. With regard to the colon, PGE_2 has been of major interest, because this prostanoid is reported to be the most abundant in both normal human gastric and colonic tissues (11–13) or to rank second in abundance behind 6-keto-prostaglandin $F_{1\alpha}$ (14, 15). PGE_2 has a direct regulatory effect on both humoral and cellular immunity by functioning as a feedback inhibitor of lymphokine production, T-cell proliferation, and macrophage and natural killer cell cytotoxicity (16–18). Inhibition of PGE_2 synthesis might enhance immune responsiveness and indirectly result in increased killing of tumor cells (19). In contrast, stimulation of PGE_2 synthesis resulting from exposure to tumor-promoting agents such as phorbol esters is thought to contribute to the tumorigenic effect of these agents via inhibition of normal immune function (20). In human colonic tumors, the PGE_2 concentration is increased significantly relative to the concentration in adjacent normal mucosa (21–23). However, this increase may be due to enhanced synthesis by infiltrating monocytes rather than increased production by tumor cells (24).

Although data indicate that aspirin protects against colonic cancer, there are no data regarding the effect of aspirin on prostaglandin synthesis in the human colonic mucosa. In this study, we examined the PGE_2 concentration in colonic mucosa and the quantity released in medium containing colonic mucosal explants obtained from subjects prior to and following administration of 325 and then 650 mg of aspirin for 2 months for each dosage. Supplementation of culture medium with arachidonic acid results in increased synthesis of LTB_4 in the human intestinal cell line CaCo-2 (25). For the present study, we also determined whether inhibition of cyclooxygenase results in a shunting of arachidonic acid through the lipoxygenase-dependent pathway, as evidenced by an increase in LTB_4 production in colonic mucosal tissue.

MATERIALS AND METHODS

Subjects. A total of 17 subjects were recruited for the study (age, 65.6 ± 13.6 years; range, 32–85 years; sex, 12 males and 5 females). All subjects had a surgical resection to remove a colon tumor (Dukes A or B) within 5 years prior to entry into the study. Subjects had received no chemotherapy or radiation as part of their treatment and had undergone colonoscopy within 2 years prior to entering the study to establish an absence of additional polyps or tumors. Subjects had a standard medical history and physical examination and submitted samples for fecal hemoccult analysis, serological analysis (liver function and complete blood cell, platelet, and differential cell counts) and routine urinalysis prior to entry into the study. Bleeding times were measured prior to administration of aspirin and 7, 60, and 120 days after commencing aspirin consumption. Subjects were asked to maintain their normal diet and avoid vitamin supplements and were provided a list of more than 100 NSAIDs and NSAID-containing products to avoid during their participation. Study participants signed an informed consent document approved by the Institutional Review Board at Loyola University prior to their participation.

Study Design. Subjects qualifying for inclusion in the study on the basis of their medical history and results from clinical examinations underwent an unprepped flexible sigmoidoscopy during which 12–15 mucosal samples were obtained by pinch biopsy 15–20 cm from the anal verge. Subjects were then provided with 60 tablets each containing 325 mg aspirin (LNK International, Hauppauge, NY) and were instructed to take one tablet each morning for 60 days. After 7 days, subjects returned to clinic to determine bleeding time. After the initial 60-day period, subjects had bleeding times determined and again underwent sigmoidoscopy, during which an additional 10–15 biopsy samples were obtained. Subjects were then provided with an additional 120 aspirin tablets (325 mg) and instructed to consume two tablets each morning. After this second 60-day period, additional biopsy samples were obtained, and bleeding time was again determined. Of the 17 subjects who began the study, 5 dropped out due to gastric and/or intestinal discomfort during the first 2 weeks of this second 60-day period, and 2 declined further participation.

The mucosal biopsy samples were removed from the biopsy forceps and snap frozen in N_2 or transferred to culture medium, which consisted of RPMI containing 10% FCS, 5 mM glutamine, and 50 μg gentamicin/ml at 4°C (Life Technologies, Inc., Gaithersburg, MD). In a tissue culture hood, individual samples were placed in wells containing 200 μl of medium alone or medium plus aspirin (1 μM final) in a 96-well culture plate. Tissue was incubated at 37°C for 4 h in a humidified atmosphere containing 5% CO_2. Medium was recovered from wells and microfuged for 1 min to pellet any cellular debris, and the supernatant was recovered for storage at −70°C. The tissue was also recovered and placed in 100 μl of 0.85% NaCl for storage at −70°C. The PGE_2 and LTB_4 concentration in medium and tissue was determined subsequently in duplicate.

Assays and Data Analysis. PGE_2 and LTB_4 concentrations were determined using enzyme immunoassays according to the manufacturer's protocols (Cayman Chemical, Ann Arbor, MI). PGE_2 concentration in medium is reported for all 17 study subjects for samples obtained at 0 and 2 months and 9 subjects for samples obtained at 4 months. The LTB_4 concentration in medium relative to the assay sensitivity required that a considerable quantity of sample be used for quantitative analysis. This quantity was sufficiently great as to preclude additional analysis if values from the duplicate analyses were highly discrepant (>20%). Thus, values for LTB_4 concentration in medium for tissue samples obtained at 0 and 2 months are for 15 subjects, and those for 4-month samples are for 7 subjects. Similarly, the sample volume needed to determine tissue PGE_2 concentration precluded repeat analysis, and thus data are provided for samples from 14 of the 17 participating subjects. Protein concentrations for tissue samples homogenized in PBS containing 0.1% Triton X-100 were determined using the Bradford protein assay (Bio-Rad, Hercules, CA).

Data are expressed as mean ± SD. Data were analyzed using a two-sided Wilcoxon matched-pairs signed-ranks test, and significance is reported for data with $P < 0.05$.

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Table 1 PGE_2 and LTB_4 concentration in media from samples incubated for 4 hours
Samples were obtained prior to and following 2 and 4 months of aspirin administration.[a]

Sample	Month	*N*	PGE_2	*N*	LTB_4
Medium	0	17	2.45 ± 2.03	15	6.87 ± 4.24
Medium + ASA	0	17	1.24 ± 0.49	15	6.91 ± 4.34
Medium	2	17	1.02 ± 1.19	15	9.57 ± 7.98
Medium + ASA	2	17	0.55 ± 0.46	15	6.33 ± 3.55
Medium	4	9	0.73 ± 0.27	7	10.20 ± 5.74
Medium + ASA	4	9	0.66 ± 0.47	7	9.10 ± 6.88

[a] Values for PGE_2 are ng/mg tissue protein and for LTB_4 are pg/mg protein.

Table 2 PGE_2 and LTB_4 concentration in colonic tissue obtained prior to and following consumption of aspirin for 2 (325 mg daily) and 4 (650 mg daily) months[a]

Month	*N*	PGE_2	*N*	LTB_4
0	14	1.17 ± 0.80	15	6.36 ± 2.63
2	14	0.59 ± 0.81	15	5.54 ± 3.80
4	8	0.17 ± 0.08	7	5.92 ± 3.46

[a] Values for PGE_2 are ng/mg tissue protein and for LTB_4 are pg/mg protein.

RESULTS

Preliminary histological analysis of tissue integrity as a function of time in medium revealed that after 4 h there was a progressive loss of normal crypt structure. After 4 h of incubation the tissue was intact and microscopically similar to tissue that had been fixed immediately upon recovery from subjects. As a measure of compliance, we determined bleeding time at 7, 60, and 120 days after commencing aspirin administration. With the exception of two subjects, one of whom had a decreased bleeding time at 7 days only and the second with a decreased bleeding time at both 60 and 120 days, bleeding times were increased for all subjects during aspirin administration relative to pre-aspirin values.

PGE_2 concentration in medium for samples collected after 2 months of administration of aspirin was reduced significantly relative to samples collected pre-aspirin ($P < 0.05$; Table 1). After an additional 2 months with administration of 650 mg aspirin/day, PGE_2 concentration in medium was reduced further relative to values for samples obtained at 0 ($P < 0.05$) and 2 months, but the difference between 2- and 4-month values was not significant. The tissue concentration of PGE_2 was also decreased significantly in samples from both 2 and 4 months compared to values from 0-month samples ($P < 0.05$ for both 2- and 4-month values). The difference in values was significant for samples obtained from 2 *versus* 4 months (Table 2).

LTB_4 concentration in medium was increased, but not significantly in samples collected at 2 months as compared to 0-month samples. At 4 months, LTB_4 concentrations were again increased compared to 0 and 2 months, but values were not significantly different from those obtained for 2-month samples. However, relative to pre-aspirin values, LTB_4 was increased significantly in samples collected at 4 months ($P < 0.05$; Table 1). The concentration of LTB_4 in tissue was not changed significantly in samples obtained at either 2 or 4 months relative to samples collected at 0 months (Table 2).

Addition of aspirin to culture medium resulted in decreased production of PGE_2 relative to medium containing no aspirin for samples collected at 0 months ($P < 0.01$), 2 months (not significant), and 4 months (not significant). The addition of aspirin to medium did not significantly alter LTB_4 production relative to medium containing no aspirin. In fact, for samples collected at 2 and 4 months, the LTB_4 concentration in medium decreased slightly as compared to medium samples that had not been supplemented with aspirin (Table 1).

DISCUSSION

Aspirin appears to prevent colon cancer in humans at low doses, but recent data suggest that the chemopreventive effect may require chronic and long-term administration (1–5, 8). The mechanism by which aspirin prevents colon cancer and whether this prevention results from a reduction in the events that initiate or cause the progression of colon cancer are not known. The primary known effect of aspirin is irreversible inhibition of cyclooxygenase, resulting in decreased prostaglandin production (26). However, there are data that suggest that NSAIDs in general may prevent cancer by mechanisms that do not involve inhibition of prostanoid production. For example, sulindac sulfone, which is metabolized from sulindac, lacks cyclooxygenase-inhibitory activity yet significantly inhibits mammary tumor formation in rats injected with 1-methyl-1-nitrosourea (27). In addition, sulindac sulfone inhibits growth and induces apoptosis of the human colonic tumor cell line HT-29, but these two events are independent (28). Other NSAIDs also inhibit cell growth *in vitro* but at concentrations that far exceed those required for inhibition of cyclooxygenase activity (29, 30). Thus, a correlation linking growth inhibition and by inference tumor formation to an inhibition of cyclooxygenase activity is lacking.

Although other mechanisms may help explain the cancer-preventive effect of aspirin, an investigation of prostaglandin production and ultimately the resulting effect of reduced production on immune cell proliferation and cytotoxicity as well as lymphokine synthesis in the colon is an obvious area of primary interest. Recently, Sano *et al.* (31), using immunohistochemistry, showed that expression of the inducible cyclooxygenase COX-2 is enhanced markedly in colon tumor cells and associated inflammatory, vascular, and connective tissue as compared to normal human tissue. This result has been confirmed using *in situ* hybridization by Kutchera *et al.* (32). NSAIDs including aspirin may function as chemopreventives by limiting the effect of this increase in COX-2 expression in colon tumors. Recently, Hinson *et al.* (33) reported

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that PGE_2 induces interleukin 6 production, which is required for the growth of some tumors. These authors suggest that inhibition of COX-2 by NSAIDs may influence tumorigenesis indirectly by inhibition of interleukin 6 production.

There have been no data indicating that aspirin administered orally inhibits cyclooxygenase activity as evidenced by a decrease in prostaglandin production in the intact human colon. Piroxicam has been shown to reduce PGE_2 concentration in a dose-dependent manner in rectal mucosal biopsies obtained from patients with a prior adenomatous polyp, but data regarding other NSAIDs are lacking (34). The data from the current study indicate that aspirin inhibits production of PGE_2 in the colonic mucosa and that a dosage of 325 mg/day is sufficient to achieve considerable inhibition. The 650 mg/day dose further decreased PGE_2 synthesis in tissue relative to that achieved with 325 mg of aspirin. We did observe a decrease in PGE_2 in medium by increasing the dosage from 325 to 650 mg, but the decrease was not significant. The use of the higher dose may not be warranted, particularly in view of the rather abrupt increase in complaints of gastric discomfort with the 650-mg dose. Our dose-response analysis does not include the lower 80-mg dosage, which is referred to commercially as baby aspirin. We assume that an 80-mg dose would decrease PGE_2 production fractionally relative to the 325-mg dose effect, but additional study is necessary to establish this extent of this effect. The effect of the 80-mg dose on PGE_2 production in the colonic mucosa has particular relevance, given that this dosage is often recommended for chronic consumption in individuals with specific cardiac symptoms.

We hypothesized that inhibition of cyclooxygenase would result in a shunting of arachidonic acid through the 5-lipoxygenase pathway as evidenced by an increase in LTB_4 production. LTB_4 is a prominent product of neutrophils but is also synthesized in the normal colonic mucosa (35). An increase in LTB_4 concentration results from addition of arachidonic acid to CaCo-2 cells *in vitro*, suggesting that increased arachidonic acid resulting from inhibition of cyclooxygenase may increase LTB_4 production (25). In contrast, synthesis of LTB_4 and other products of the lipoxygenase pathway is not altered in rat mucosa following treatment with aspirin (36). Our results indicate that inhibition of cyclooxygenase as reflected by a decrease in PGE_2 production does not effect LTB_4 synthesis in the human colon. The quantity of LTB_4 in colonic mucosa is greater than 300-fold less than the concentration of PGE_2, and in samples obtained from subjects consuming aspirin for 4 months, it is less than 50-fold the PGE_2 concentration. PGE_2 and LTB_4 are both proinflammatory but differ in regard to their primary cellular targets. LTB_4 is produced primarily by and is a chemoattractant for neutrophils and would thus be in a higher concentration in inflamed *versus* uninflamed tissue (37). The effect of aspirin on LTB_4 production may reflect the extent of local inflammation. Although we believe that the tissue obtained from subjects was noninflamed, we do not know how much of the LTB_4 detected originated in neutrophils and how much was synthesized by epithelial cells in different samples. Small differences in the number of neutrophils may have overshadowed the relatively lower production of LTB_4 production in epithelial cells and the sensitivity of this source of LTB_4 to aspirin. Although we did observe a significant increase in LTB_4 concentration after 4 months aspirin administration as compared to values from pre-aspirin samples, the fact that a similar increase was not evident in tissue samples and that addition of aspirin to culture medium did not increase LTB_4 concentration strongly suggests that aspirin does not have an appreciable effect on LTB_4 production in colonic mucosal tissue.

Our study subjects were older cancer patients who had undergone a colonic resection within the previous 5 years. We reported recently that the colonic mucosa of these subjects does not differ in regard to three independent indices of cellular proliferation from the colonic mucosa of normal subjects (38). We suspect that PGE_2 and LTB_4 production by the colonic mucosa of normal subjects would not differ from that of cancer subjects, but additional study into this as well as into the effect of age on PGE_2 production in the colonic mucosa is needed. We have demonstrated that results obtained in the *ex vivo* experiments were comparable in regard to relative concentrations of PGE_2 and LTB_4 to results obtained from tissue samples that were flash frozen following procurement from the study subjects. The use of colonic explants in this manner should be useful in determining other possible effects of aspirin as well the effect of other NSAIDs in cancer prevention.

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