Effect of Subacute Ibuprofen Dosing on Rectal Mucosal Prostaglandin E_2 Levels in Healthy Subjects with a History of Resected Polyps[1]

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Abstract

Nonsteroidal antiinflammatory drugs are among the most promising chemopreventive agents for colorectal cancer. Although the mechanism by which nonsteroidal antiinflammatory drugs exert such effects remains to be further characterized, their best known pharmacological effect is inhibition of prostaglandin synthetase, which leads to decreases in tissue prostaglandin levels. We conducted a randomized, double-blind, controlled study to examine the effect of daily ibuprofen treatment on the rectal mucosal prostaglandin E_2 (PGE_2) levels in healthy subjects with a history of resected polyps. Study participants ($n = 27$) completed a 2-week run-in period and were then randomized to take a single, daily dose of ibuprofen (300 or 600 mg) or of a placebo for 4 weeks. Rectal biopsy specimens were taken before and after the run-in period and at 2 and 4 weeks after the ibuprofen/placebo treatment. Notably large between- and within-subject variability in the rectal mucosal PGE_2 content was seen. The changes in PGE_2 levels after ibuprofen/placebo treatment correlated with the baseline PGE_2 content. After adjustment of the baseline values, 2 weeks of 300 mg/day of ibuprofen treatment resulted in significantly more suppression of PGE_2 levels than that observed after the placebo treatment (55% *versus* 22% suppression from baseline; $P = 0.033$). Although other ibuprofen treatment schedules and doses appeared to result in suppression in the PGE_2 levels, the suppression was not statistically significant because of the large variability in this measurement. Because lower doses are associated with fewer adverse effects, a dose of 300 mg of ibuprofen/day should be considered for future Phase II chemoprevention studies. Stratifying study participants, based on their baseline PGE_2 levels and inclusion of a larger number of study subjects, are recommended for future trials where the rectal mucosal PGE_2 level is to be used as a surrogate end point biomarker.

Introduction

Cancer of the colon is the second most prevalent malignancy and the second leading cause of cancer death in the United States. There is strong evidence that adenomatous colon polyps are precursor lesions of colon cancer. Such polyps are found in ~10% of adults with incidence increasing to as high as 50% in persons >70 years of age (1). Despite improvements in surgical techniques and the development of active adjuvant chemotherapy for advanced disease, 5-year cure rates of advanced disease remain low.

The most promising strategies to reduce colorectal cancer mortality involve developing improved screening methods and novel preventive approaches. Among potential chemopreventive agents for colorectal cancers, promising results have been shown with the use of NSAIDs.[3] A variety of NSAIDs have been shown to have reduced the formation of both colon adenomatous polyps and cancers in experiments where animals had been administered known carcinogens (2–8). NSAIDs also inhibited the growth and clinical expression of transplanted tumors and metastatic cancer spread in animal models (9–12), and they potentiated the antitumor effects of immunotherapy, radiotherapy, and anticancer drug treatment (13–15). Tumor and cell culture studies (16, 17) have shown that NSAIDs alter the cycle and proliferation of colon cancer cells. These preclinical data consistently support the use of NSAIDs for inhibition of carcinogenesis in the colon.

Many epidemiological studies (18–25) have examined the relationship between aspirin and other NSAID use and colorectal cancer. Most of these studies show a marked decrease in the relative risk (40–50%) of this cancer among continuous users. In contrast, both randomized and observational analyses from the Physicians' Health Study suggested that there is no association between the use of aspirin and the incidence of colorectal cancer (26, 27). Perhaps the most convincing data concerning the potential role of NSAIDs as chemopreventive agents for colorectal adenomas and ultimately cancer come from reports that sulindac promotes regression and inhibits the recurrence of adenomatous colon polyps in patients with familial adenomatous polyposis (28–32).

The mechanism(s) by which NSAIDs may reduce the risk of colon cancer is not known, but it may be related to the effect of these agents on colorectal mucosal prostaglandin levels because these agents are known to inhibit cyclooxygenase and to reduce prostaglandin synthesis. Prostaglandins have been shown to significantly affect cell proliferation and tumor

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[3] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; BMI, body mass index.

growth (33, 34). We have reported that the PGE_2 content of colon adenocarcinoma and adenomatous polyps was higher than that in normal adjacent colonic mucosa (35). Similarly, the levels of PGE_2 in colon cancer samples were found by another research group to be elevated in comparison with histologically normal mucosal samples 5–10 cm away from the tumor (36). Nevertheless, other potential mechanism(s), such as induction of apoptosis through nonarachidonic acid pathways, have been proposed to explain the cancer chemopreventive activity of NSAIDs (37).

Although observational and laboratory studies suggest that the incidence of colorectal cancer may be reduced by the use of NSAIDs, further clinical research is needed to determine the effects of individual NSAIDs and the appropriate prophylactic dose and duration of treatment. Because ibuprofen is a commonly used NSAID with an excellent safety record, we performed a Phase I study of this drug in healthy subjects with a history of resected polyps. The effect of daily oral doses of ibuprofen on PGE_2 levels in rectal mucosal biopsies was determined.

Patients and Methods

Participants. Healthy human subjects were recruited through media announcements and referral by local gastroenterologists. To be eligible, the participants were required to be ≥18 years of age, to have had colorectal adenomatous polyps removed within 5 years before study entry, to be able to give informed consent, to be in performance status 0–1 (determined by Southwest Oncology Group Performance Status Criteria), to have normal liver and renal function, and to have adequate dietary intake of calories and protein (determined by the Arizona Cancer Center adaptation of the National Cancer Institute Food Frequency Questionnaire). Participants were excluded if they were pregnant; had a history of previous invasive cancer, severe metabolic disorders, or other life-threatening acute or chronic diseases; were taking medications within 1 week of the study; and had weight loss >10% in the 6 months preceding study entry. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

Study Design. All study participants underwent a 2-week placebo run-in period for compliance evaluation. After the successful completion of the run-in period, 27 study participants were randomly assigned to receive one of the following treatments on a once daily schedule for 4 weeks: placebo, 300 mg/day of ibuprofen, and 600 mg/day ibuprofen. Plasma and rectal mucosal biopsies were collected before the run-in period, at the end of the run-in period, after 2 weeks of ibuprofen/placebo treatment, and after 4 weeks of ibuprofen/placebo treatment. Study participants were instructed to fast for a minimum of 6 h before sample collection. All blood samples and rectal biopsies were collected at ~2–4 h after ingestion of ibuprofen or placebo. Plasma ibuprofen concentrations and PGE_2 content in rectal mucosal biopsies were determined (see below).

Rectal Biopsy Sample Collection. Participants were prepped using two 150-ml tap water enemas 30 min to 2 h before the procedure. After the insertion of the sigmoidoscope into the rectum, biopsies were taken perpendicularly to the mucosal surface from the upper half of the rectum (12–18 cm from the anus). Because the amount of endogenous PGE_2 can be affected by the depth of the biopsy (38), routine-sized forceps were used to ensure continuity of biopsy depth into the mucosa. Samples were immediately placed in cryovials containing aqueous indomethacin (5 μg/ml) and then snap frozen in liquid nitrogen. The cryovials were labeled to indicate the order in which the biopsies were collected. All tissue samples were stored in liquid nitrogen until the time of analysis.

Analysis of PGE_2 Content in Rectal Mucosal Biopsies. PGE_2 content in rectal mucosa was determined using procedures described previously (39). Briefly, after thawing, the first two biopsies collected were pooled and placed into 1 ml of 0.05 M Tris-HCl buffer (pH 7.4) containing 5 μg/ml of indomethacin. Because the PGE_2 levels can be affected by the depth of the biopsy (38), the biopsies from the first two collections were pooled to minimize a portion of the variability and to give an accurate depiction of the mucosal PGE_2 levels. Tissue was homogenized in a siliconized glass tissue grinder for 30 s. An aliquot of the homogenate was removed for protein concentration determinations. One hundred % ethanol (2 ml) was added to the remaining homogenate and allowed to stand for 5 min on ice. Distilled water was added to the homogenate to result in a final ethanol concentration of 15%. The samples were centrifuged at 4°C for 10 min at 3000 rpm. The supernatant was removed and the pH was adjusted to 3.0 with 0.25 M HCl. The sample was then applied to a C_{18} silica column previously washed with 20 ml of 100% ethanol followed by 20 ml of distilled water. The column was rinsed with 20 ml of a 15% ethanol solution followed by 20 ml of petroleum ether. PGE_2 was gravity-eluted with 10 ml of methyl formate. The methyl formate was divided into four equal 2.5-ml aliquots, dried under nitrogen, and stored at −80°C. Samples were reconstituted in 0.25 ml of assay buffer and assayed for PGE_2 content using a Dupont ^{125}I- PGE_2 RIA kit (Dupont New England Nuclear, Boston, MA).

Ibuprofen Assay. Plasma ibuprofen concentrations were analyzed by high-performance liquid chromatography using a method of Shah and Jung (40) with minor modifications. Briefly, mefenamic acid (Sigma, St. Louis, MO) dissolved in 50% acetonitrile, 50% methanolic-HCl (0.1 N) to a concentration of 10.0 mg/ml was used as the internal standard. Two hundred and fifty μl of the internal standard solution were mixed with 100 μl of plasma samples or blank plasma spiked with ibuprofen standards. After vortexing, the mixtures were centrifuged at 10,000 rpm for 20 min. Fifty μl of the resulting supernatant were injected onto a C_{18} column (μBondapak, 3.9 × 300 mm, Waters Associates, Milford, MA). A mobile phase consisting of methanol, acetonitrile, water, and 85% phosphoric acid in a ratio of 10:36:54:0.05 at a flow rate of 1.5 ml/min was used. The UV absorbance of the effluent was monitored with a Hewlett-Packard 1040A Diode-Array Detector at 196 nm.

Data Analysis. Plasma ibuprofen levels were transformed logarithmically before being subjected to statistical analyses. The statistical comparisons of the plasma ibuprofen concentrations after different ibuprofen treatments were performed using the ANOVA for repeated measurements. In this analysis, dose and duration of treatment were included as the main effects. A $P < 0.05$ for the main effects was considered to be statistically significant. Bonferroni's t test was used for the pairwise multiple comparisons.

Changes in logarithmically transformed PGE_2 levels at weeks 2 and 4 from those at baseline were computed as $\ln(PGE_2)_{posttreatment} - \ln(PGE_2)_{baseline}$. This calculation gave rise to values equivalent to the logarithmically transformed ratio of the posttreatment PGE_2 levels:PGE_2 levels at baseline ($\ln((PGE_2)_{posttreatment}/(PGE_2)_{baseline})$). Comparisons of the changes in PGE_2 levels after ibuprofen/placebo treatment were

Table 1 Demographic data of the study participants

	Placebo	Ibuprofen 300 mg/day	Ibuprofen 600 mg/day
No. of subjects	9	9	9
No. of present smokers	1	1	1
No. of females	4	4	3
Age (year)	58 ± 13[a]	59 ± 10	62 ± 5
	(39–73)[b]	(39–70)	(54–69)
Height (cm)	167 ± 16	171 ± 11	172 ± 7
	(134–188)	(161–190)	(161–180)
Weight (kg)	88 ± 28	87 ± 24	83 ± 9
	(64–152)	(58–121)	(65–96)
BMI (kg/m^2)	31.7 ± 8.2	29.6 ± 7.8	28.3 ± 3.9
	(23–49)	(21–44)	(20–31)

[a] Mean ± 1 SD.
[b] Data range.

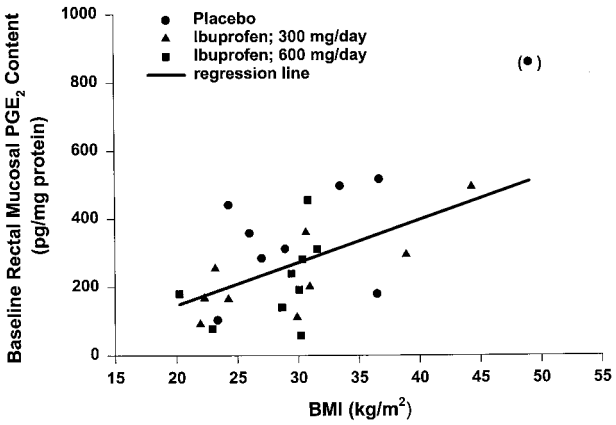

Fig. 1. Plot of the relationship between baseline rectal mucosal PGE_2 content and BMI. The baseline rectal mucosal PGE_2 content was an average of the data collected before and at the end of the run-in period. ——, a regression fit of the data ($Y = 12.5 \times X - 103.2$; $r^2 = 0.269$; $P = 0.007$) without the inclusion of the data point in parentheses.

performed using the analysis of covariance using the baseline values as the covariant.

Results

Table 1 presents the demographic data of the study participants. A total of 27 subjects (9 per group) completed the study. There were no significant differences in the demographic disposition of the participants among the three study groups. Fig. 1 illustrates the relationship between baseline PGE_2 levels and BMI. BMI was found to be a positive predictor of baseline PGE_2 levels with ($P = 0.007$) or without ($P < 0.0001$) the exclusion of results of one of the subjects with a high PGE_2 level.

Toxicities in the present study were graded according to the criteria of the World Health Organization. Both ibuprofen dose levels were well tolerated during the 28-day drug administration. Of 16 reported adverse events attributed to a possible relationship with ibuprofen, only 2 were considered to be severe. One participant in the placebo cohort reported diarrhea starting 13 days after beginning the study medication (placebo) and lasting for a period of 3 days. Symptoms improved with administration of Imodium. Another participant in the 300-mg/day cohort experienced tinnitus 26 days after beginning the study medication. This adverse event lasted in excess of 8 h, resolved without intervention, and did not recur. Both of these participants completed the study. Of the remaining 14 adverse events reported, 8 were judged to be mild and 6 were judged to be moderate.

The mean plasma concentrations of ibuprofen obtained during the study for each treatment are shown in Table 2. No detectable levels of ibuprofen were found in baseline plasma samples from subjects in the placebo and 300-mg/day groups. A very low level of ibuprofen was detected in baseline plasma samples of one subject in the 600-mg/day group. This could have resulted from self-administration of ibuprofen outside the study. After daily treatment with ibuprofen, ibuprofen was detected in all treated subjects. Dose-dependent increases in plasma ibuprofen concentration were observed as the daily dose increased from 300 mg to 600 mg (12.7 ± 4.5 *versus* 21.1 ± 6.1 μg/ml, $P < 0.05$, after 2 weeks of treatment; 13.3 ± 3.2 *versus* 24.2 ± 10.0 μg/ml, $P < 0.05$, after 4 weeks of treatment). For both the 300-mg/day and 600-mg/day treatment groups, there were no statistically significant differences between the ibuprofen plasma concentration after either 2 or 4 weeks of treatment.

The mean PGE_2 levels in rectal mucosa before and after 2 and 4 weeks of ibuprofen/placebo treatment are shown in Table 3. The changes in the rectal mucosal PGE_2 content in each individual before and after placebo/ibuprofen treatment are illustrated in Fig. 2. There was more variability in the baseline PGE_2 content between subjects in the placebo group than in those in the ibuprofen groups. After the ibuprofen treatment, a more consistent reduction in the PGE_2 levels was observed after 2 weeks of ibuprofen treatment than after placebo treatment. An additional 2 weeks of ibuprofen treatment did not seem to result in further reduction in the PGE_2 levels.

The changes in PGE_2 levels at weeks 2 and 4 of treatment, expressed as percent of the baseline values, are presented in Table 4. The changes in PGE_2 levels from baseline at week 2 were marginally affected by the baseline values when this variable was examined as the covariant ($P = 0.081$). Marginal differences were found in the changes in PGE_2 levels among different treatment groups ($P = 0.097$). After adjustment for the covariant, the suppression in PGE_2 levels from baseline after 2 weeks of 300 mg/day of ibuprofen treatment was significantly more than that observed after placebo treatment [45% of baseline (*i.e.*, 55% suppression) *versus* 78% of baseline (*i.e.*, 22% suppression); $P = 0.033$]. The changes in PGE_2 levels from baseline after 2 weeks of 600 mg/day of ibuprofen treatment were not significantly different from those observed after the placebo treatment ($P = 0.24$). No differences in the changes in PGE_2 levels from baseline after 2 weeks of 300 or 600 mg/day of ibuprofen treatment ($P = 0.29$) were found. The changes in PGE_2 levels from baseline at week 4 were significantly correlated with the baseline values when this variable was examined as the covariate ($P = 0.0005$). No significant differences were found in the changes in PGE_2 levels among the treatment groups ($P = 0.13$).

Discussion

NSAIDs are among the most promising chemopreventive agents for colorectal cancer. Although the mechanism by which NSAIDs exert chemopreventive effects remains to be further characterized, the best known pharmacological effect of NSAIDs is inhibition of prostaglandin synthetase, leading to decreases in tissue prostaglandin levels. The effects of NSAIDs on colorectal mucosal PGE_2 levels have been examined in a number of recent investigations. Ruffin *et al.* (41) reported the

Table 2 Mean plasma ibuprofen levels (μg/ml) before and after daily ibuprofen treatment

Ibuprofen dose (mg/day)	Before the placebo run-in period	At the end of the placebo run-in period	After 2 wk of ibuprofen treatment	After 4 wk of ibuprofen treatment
0 (placebo)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
300	0 ± 0	0 ± 0	12.7 ± 4.5	13.3 ± 3.2
600	0.17 ± 0.52[a]	0.20 ± 0.60[a]	21.1 ± 6.1[b]	26.3 ± 8.0[b]

[a] Resulted from ibuprofen levels seen in one individual.
[b] Significantly different from subjects taking 300 mg/day ibuprofen; $P < 0.05$.

Table 3 Mean rectal mucosal PGE_2 content (pg/mg protein) before and after ibuprofen treatment

Ibuprofen dose (mg/day)	Averaged baseline (before and at the end of the run-in period)	After 2 wk of treatment	After 4 wk of treatment
0 (placebo)	394.7 ± 221.8	257.4 ± 180.9	178.2 ± 91.5
300	237.9 ± 128.6	100.2 ± 91.5	98.6 ± 41.4
600	215.6 ± 123.8	133.1 ± 123.9	115.1 ± 160.8

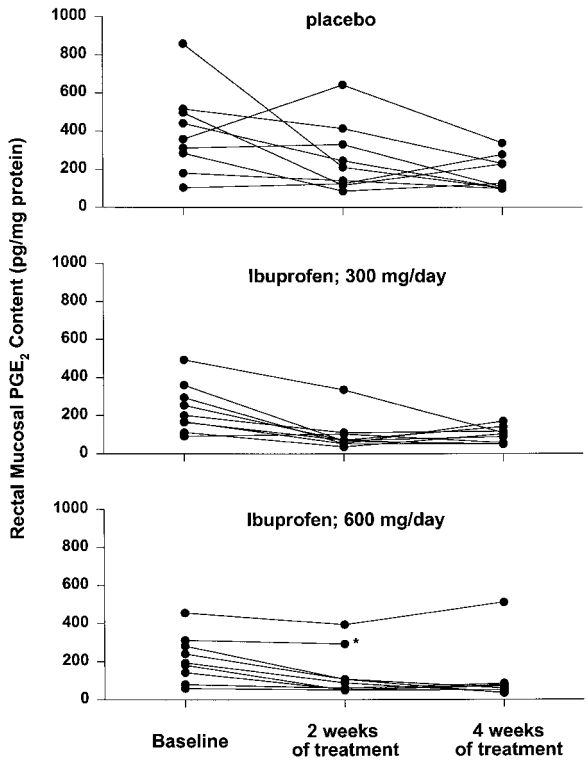

Fig. 2. Plot of the rectal mucosal PGE_2 content in each individual subject before and after placebo/ibuprofen treatment. *, the data after 4 weeks of ibuprofen treatment were not available.

effect of a daily aspirin dose on the colorectal mucosal PGE_2 levels in healthy men and women. Significant suppression of PGE_2 levels (by 23.7%) was observed after 14-day treatment with doses as low as 81 mg/day. In colectomized patients with familial adenomatous polyposis, sulindac maintenance treatment for 48 weeks reduced the PGE_2 content from 2788.8 ± 4488 pg/mg protein (baseline) to 1311.2 ± 1418.1 pg/mg protein (after treatment; Ref. 32). Because of the variability of the PGE_2 values, the reduction in colorectal mucosal PGE_2 content in this study was not statistically significant. In a smaller study in four patients with familial adenomatous polyposis, sulindac daily treatment for 3 months resulted in decreases in the PGE_2 content from 4832 ± 454 to 2930 ± 1820 pg/mg protein (42). Interpatient differences in response to sulindac were evident, with changes from +19% to −89%. In our study, notably large between- and within-subject variability was seen in the PGE_2 content. The changes in rectal mucosal PGE_2 levels after ibuprofen/placebo treatment were found to correlate with the baseline PGE_2 content. After the appropriate adjustment of the baseline values, 2 weeks of 300 mg/day of ibuprofen treatment was shown to result in significantly more suppression in PGE_2 levels than that observed after the placebo treatment (55% *versus* 22% suppression from baseline). Although other ibuprofen treatment schedules and doses appeared to result in suppression in the PGE_2 levels, the suppression was not statistically significant because of the large variability in this measurement.

Table 4 The PGE_2 levels after 2 or 4 weeks of ibuprofen/placebo treatment, expressed as percent of the baseline values ($100 \times ((PGE_2)_{posttreatment}/(PGE_2)_{baseline})$)

Ibuprofen dose (mg/day)	After 2 wk of treatment	After 4 wk of treatment
0 (placebo)	78 ± 52	69 ± 78
300	45 ± 30[a]	51 ± 28
600	59 ± 26	60 ± 46

[a] Changes in logarithmically transformed PGE_2 levels were significantly different from those after placebo treatment (see data analysis section for details); $P = 0.033$.

In this study, the baseline PGE_2 levels in subjects assigned to the 300-mg/day and the 600-mg/day study groups were similar. However, the baseline PGE_2 levels in subjects in the placebo control group were more variable and were found to be significantly higher than those in treatment group subjects (394.7 ± 221.8 *versus* 237.9 ± 128.6 *versus* 215.6 ± 123.8 pg/mg protein for placebo, 300 mg/day, and 600 mg/day, respectively). This discrepancy was considered to be unrelated to assay variability because reanalysis of sample specimens showed similar results. It is not known what variables may have contributed to the higher baseline PGE_2 levels in the control group because subjects were randomly assigned to one of the three study groups and there were no significant differences in the demographic disposition of the participants among the study groups (see Table 1). Recently, we have shown lower levels of physical activity or high BMI values to be associated with higher rectal mucosal PGE_2 concentrations (43). Similar correlation between BMI values and PGE_2 levels was observed in this study (see Fig. 1). The BMI ranged from 23 to 49, 21 to 44, and 20 to 31 kg/m^2 in the placebo, ibuprofen, 300 mg/day, and ibuprofen, 600 mg/day, groups, respectively. There were no statistically significant differences in the BMI values among

study groups, although more subjects in the placebo group had BMIs at the higher end of the range. This difference could have contributed, at least in part, to the group differences in the baseline PGE_2 values. Because we did not determine baseline physical activity levels in the present study, the contribution of this variable to the differences in the baseline PGE_2 levels among the study groups is not known. The unexpected higher and more variable baseline PGE_2 values in the placebo group probably prevented us from observing a more statistically significant treatment effect. In future prospective studies, stratifying the treatment groups based on the baseline PGE_2 levels before randomization could help better balance the subjects in the control and treatment groups.

Significant between-subject and within-subject variability in the colorectal mucosal PGE_2 content was evident in our study and in other reported trials. Notable variability in the PGE_2 content was also seen among different research groups, with values ranging from 30 to 130000 pg/mg protein (32, 41, 42, 44). This striking variability could be related to the different analytical methods used for PGE_2 measurements, different methods used in collecting the biopsies, different colorectal sites where the biopsies were collected, and the health status of the study subjects. In our previous study, we found that mucosal biopsies obtained from the same patient within a small region of the rectum may exhibit a wide range of prostaglandin content (coefficient of variation of 38%) despite maintaining a relatively consistent protein content (39). This variability seems to be related to factors other than PGE_2 assay precision because the coefficient of variation was $<10\%$ for repeat analyses of the same homogenate obtained from the same patient (39).

In the present study, there was a clear dose-dependent increase in ibuprofen plasma concentrations. It is therefore assumed that tissue ibuprofen concentrations were also increased at a higher dose. However, the 600 mg/day dosing regimen did not seem to provide an additional benefit in suppressing the mucosal PGE_2 levels. Because of its short elimination half-life (around 90 min after a 200 mg dose), ibuprofen administered once a day should result in minimal accumulation of plasma ibuprofen levels after chronic administration. Conforming with such a prediction, our results showed that regardless of dose, ibuprofen plasma levels were similar between samples collected after 2 weeks and 4 weeks of dosing. Furthermore, the plasma ibuprofen concentrations observed in this study were consistent with the concentration range observed after single dose administration reported in the literature (45).

Historically, ibuprofen has been one of the safest NSAIDs available. Meta-analysis of controlled epidemiological studies of various NSAIDs and gastrointestinal complications such as hemorrhage or perforation indicate that ibuprofen ranked lowest or equal to lowest for risk in 10 of 11 studies analyzed (46). Another study of NSAID overdoses reported to poison centers in the United States in a 2-year period determined that symptoms of overdose with ibuprofen were unlikely after ingestion of $\leq$100 mg/kg and were usually not life-threatening unless $>$400 mg/kg was ingested (47).

In summary, our data suggest that daily ibuprofen treatment for 2 or 4 weeks caused variable, but in some cases significant suppression of rectal mucosal PGE_2 levels compared to placebo-treated controls. The suppression in PGE_2 content in rectal mucosal biopsies was not different between the two ibuprofen doses (600 *versus* 300 mg) used in the study or at 2 *versus* 4 weeks of administration. Because lower doses are associated with fewer adverse effects, a dose of 300 mg of ibuprofen/day should be considered for future Phase II chemoprevention studies. Because of the large between- and within-subject variability in the rectal mucosal PGE_2 content, stratifying study participants based on their baseline PGE_2 levels before randomization and inclusion of a larger number of study subjects are recommended for future trials wherein the rectal mucosal PGE_2 level is to be used as a surrogate end point marker.

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