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Esomeprazole and 325 mg/d Aspirin Reduce Tissue Concentrations of Prostaglandin E2 in Patients with Barrett's Esophagus

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Abstract

Background & Aims—Proton pump inhibitors (PPIs) and nonsteroidal anti-inflammatory drugs might prevent esophageal adenocarcinoma in patients with Barrett’s esophagus (BE), but there are limited data from clinical trials to support this concept. We conducted a randomized, double-blind, placebo-controlled phase II trial to assess the effects of the combination of aspirin (3 different doses) and esomeprazole on tissue concentrations of prostaglandin E2 (PGE2) in patients with BE with no dysplasia or low-grade dysplasia.

Methods—Participants were recruited through the multi-center Cancer Prevention Network and randomly assigned to groups that were given esomeprazole (40 mg, twice daily) in combination with an aspirin placebo (once daily) (Arm A; n=42), with 81 mg aspirin (once daily) (Arm B; n=63), or with 325 mg aspirin (once daily) (Arm C; n=63) for 28 days. We collected esophageal biopsies before and after the intervention period, to determine the absolute change in mean concentrations of PGE2 (the primary endpoint).

Results—Based on data from 114 patients, baseline characteristics were similar among groups. The absolute mean tissue concentrations of PGE2 was reduced by 67.6 ± 229.68 pg/mL in Arm A, was reduced by 123.9 ± 284.0 pg/mL in Arm B ($P=.10$ vs Arm A), and was reduced by 174.9 ± 263.62 pg/mL in Arm C ($P=.02$ vs Arm A).

Conclusions—In combination with esomeprazole, short-term administration of higher doses of aspirin, but not lower doses or no aspirin, significantly reduced tissue concentrations of PGE2 patients with BE with either no dysplasia or low-grade dysplasia. These data support further evaluation of higher doses of aspirin and esomeprazole to prevent esophageal adenocarcinoma in these patients.

Keywords

esophageal cancer; NSAIDs; inflammation; esophagus

INTRODUCTION

Barrett’s esophagus (BE) is an acquired condition that affects about 1 in 20 United States adults.¹ Esophageal adenocarcinoma, which has become increasingly common in developed countries over the past four decades, is strongly associated with BE.² Based on currently available data, the annual incidence of esophageal adenocarcinoma in Barrett’s esophagus patients is estimated to range from approximately 0.3 to 0.5 percent.³⁻⁵ Unfortunately, the five-year survival rate for esophageal adenocarcinoma remains dismal (approximately 17%).⁶

Current strategies for improving clinical outcomes among patients with esophageal adenocarcinoma are largely focused on identifying malignant disease at an early, potentially curable stage. This could be accomplished through widespread adoption of endoscopic screening and/or surveillance programs. However, existing early detection guidelines endorsed by major subspecialty societies are inherently expensive, with a relatively limited evidence base.⁵ Thus, further evaluation of novel approaches to esophageal cancer risk reduction among BE patients is urgently needed.

Extensive preclinical data suggest that non-steroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, may play a protective role against the development of esophageal

adenocarcinoma by inhibiting the cyclooxygenase 1 and 2 (COX-1 and COX-2) enzymes, which regulate prostaglandin E₂ (PGE₂) production⁷⁻¹². Upregulation of PGE₂ has been associated with resistance to apoptosis, increased angiogenesis, and enhanced invasion in Barrett's mucosa and other gastrointestinal neoplasia, thereby providing a strong biologic rationale for assessing PGE₂ modulation as a surrogate endpoint biomarker for phase II trials.⁷⁻¹²

One proposed mechanism for Barrett's-associated carcinogenesis is acid and/or bile salt induced COX-2 activation (with accompanying high levels of PGE₂ production), which would support the chemopreventive potential of a proton pump inhibitor (PPI) given in combination with aspirin. However, to date, the chemopreventive potential of PPI and/or NSAID therapy for Barrett's-associated esophageal adenocarcinoma remains inconclusive,⁵ and the combination approach has not been evaluated in a randomized clinical trial. For this multi-center, double-blind, placebo controlled phase II clinical trial, we hypothesized that a PPI plus higher dose aspirin would be more effective for downregulating PGE₂ than either a PPI alone or a PPI plus lower dose aspirin in BE patients.

METHODS

All aspects of the study protocol were reviewed and approved by the appropriate Institutional Review Board for human research at each participating site. Mayo Clinic in Rochester, MN, served as the coordinating research base. The Data and Safety Monitoring Board of the Mayo Clinic Cancer Center reviewed safety data every 6 months and efficacy data within 6 months of the planned interim analysis.

General Overview

Participants were recruited at 12 Cancer Prevention Network (CPN) member organizations: Cleveland Clinic, Cleveland, OH; Fox Chase Cancer Center, Philadelphia, PA; Hines Veterans Administration, Hines, IL; Kaiser Permanente Medical Group, San Francisco, CA; Kansas City VA Medical Center, Kansas City, MO; Massachusetts General Hospital, Boston, MA; Mayo Clinic, Scottsdale, AZ, Jacksonville, FL, and Rochester, MN; St. Michael's Hospital, Toronto, Canada; University Hospitals Case Medical Center, Cleveland, OH; and the University of Puerto Rico Cancer Center, San Juan Puerto Rico). In total, 120 eligible participants were enrolled between May 2007 and January 2011.

Study Participants

The target population included adult patients (≥ 18 years) with histologically-confirmed BE, defined as the presence of specialized columnar epithelium anywhere in the tubular esophagus with ≥ 2 cm of circumferential involvement and no evidence of high-grade dysplasia or cancer based on both clinical surveillance and additional research biopsies. Participants were required to have documented intestinal metaplasia with goblet cells in ≥ 4 out of 8 research biopsy samples (with ≥ 50% intestinal metaplasia) and could not have used aspirin, NSAIDs, or selective COX-2 inhibitors for ≥ 7 days during the 3 month period preceding a 28 day run-in phase. Women of childbearing potential were required to document a negative pregnancy test prior to enrollment. General exclusion criteria were: inability to abstain from aspirin, NSAID, or selective COX-2 inhibitor therapy for the duration of the trial; current or planned use of anticoagulants; prior fundoplication, bariatric surgery or any other major upper GI surgery (except cholecystectomy); history of confirmed esophageal high-grade dysplasia or cancer; history of cancer diagnosis ≥ 24 months (except non-melanoma skin cancer or cervical carcinoma-in-situ) or cancer treatment ≥ 12 months (except hormone therapy) prior to run-in; receipt of other investigational agents ≥ 3 months prior to run-in (except compounds such as multivitamins, topically-applied skin creams, etc.

with no known interaction with the study agents); history of allergic reactions attributed to compounds of similar chemical or biologic composition to the study agents or rescue medication; history of endoscopically or radiographically diagnosed peptic ulcer disease (bleeding or nonbleeding); pregnant or lactating women; or comorbidities that might limit adherence to the study protocol.

Baseline Evaluation and Run-In Phase

Following informed consent, willing participants completed a focused interview, brief questionnaire, physical exam, and peripheral blood draw for eligibility testing. Participants who met enrollment criteria entered a 28 day run-in phase, which included self-administration of esomeprazole 40 mg bid (i.e., before breakfast and dinner). Eighty percent adherence with the run-in agent, based on an agent diary and follow-up phone call at 14 (+/- 3) days, was required for advancement to randomization. A repeat blood draw and baseline endoscopy were scheduled for 28 (+/- 3) days after initiating the run-in phase.

Pre-Intervention Endoscopy

The pre-intervention endoscopy exam was performed with a therapeutic endoscope (Olympus GIF-1T140 videoendoscope or equivalent). Endoscopic landmarks including the diaphragmatic hiatus, end of the tubular esophagus as marked by the proximal margin of gastric folds, and new squamocolumnar junction were recorded. Extent of the circumferentially involved BE segment was determined using the Prague classification system.¹³ Hiatal hernia size was measured by linear distance between the end of the tubular esophagus and the diaphragmatic hiatus. Short segment BE was defined as specialized columnar epithelium lining < 3 cm of the distal esophagus, whereas long segment BE was defined as specialized columnar epithelium lining ≥ 3 cm of the distal esophagus.

Four quadrant endoscopic surveillance biopsies were obtained using Boston Scientific Radial Jaw[®] 3 forceps at 2-cm intervals along the entire length of the Barrett's epithelium. Additional biopsy specimens were obtained from any ulcer, erosion, plaque, nodule, stricture, or other luminal irregularity. All surveillance biopsies were assessed by local pathology review. Endoscopic research biopsies (up to 8) were obtained with the same forceps from a 1 cm zone of Barrett's epithelium 1 to 2 cm above the level of the lower esophageal sphincter, just above the proximal margin of the gastric folds. Research biopsies were washed for 5 seconds in PBS and divided into two aliquots, which were snap frozen in liquid nitrogen or placed in 10% neutral buffered formalin at room temperature, respectively. All formalin fixed research biopsies were assessed by the study pathologist (TCS). If high-grade dysplasia or cancer was detected in any research biopsy, two additional gastrointestinal pathologists at Mayo Clinic in Rochester, MN, were consulted. If the consensus diagnosis (including input from the local pathologist) was high-grade dysplasia or cancer, the participant was classified as a screen failure.

Findings of ulcer, erosion, plaque, nodule, stricture, or other luminal irregularity within the Barrett's segment; erosive esophagitis > LA class A; high-grade dysplasia or cancer; or inadequate Barrett's mucosa to satisfy the study endpoints (defined as < 4 out of 8 research samples with ≥ 50% intestinal metaplasia by central pathology review) excluded further study participation.

Intervention Assignments and On-Study Monitoring

Willing, eligible participants were randomly assigned to receive a 28 day intervention with one of three study agent combinations (using a 1:1.5:1.5 schema to permit increased access to an active aspirin intervention): Arm A (aspirin placebo + PPI) = aspirin 81 mg placebo qd + aspirin 325 mg placebo qd + esomeprazole 40 mg bid (n = 42); Arm B (lower dose aspirin

+ PPI) = aspirin 81 mg qd + aspirin 325 mg placebo qd + esomeprazole 40 mg bid (n = 63); and Arm C (higher dose aspirin + PPI) = aspirin 81 mg placebo qd + aspirin 325 mg qd + esomeprazole 40 mg bid (n = 63). Esomeprazole was self-administered once in the morning and once in the evening, at least one hour before a meal. Aspirin or aspirin placebo (indistinguishable from the activate agent) was administered once in the morning with a meal. Adherence was defined as 80% ingestion of the protocol-defined doses. Antacid tablets (200 mg aluminum hydroxide, 200 mg magnesium hydroxide, and 25 mg simethicone per each tablet) were provided as rescue medication for acute GERD symptoms. Use of > 12 antacid tablets per day and/or for > 14 days was noted as a protocol deviation. Adverse events were classified and graded using NCI Common Toxicity Criteria, version 3.0 (available at www.ctep.cancer.gov). Attribution of agent-related adverse events was performed by the designated medical monitor (GDZ), who was blinded to the intervention assignments.

Post-Intervention Evaluation

Participants returned at 28 (+/- 3) days post-randomization to assess adherence, concomitant medication use, and adverse events. A focused physical exam and peripheral blood draw were also performed. Post-intervention endoscopy, research biopsies, and tissue handling were performed according to the standardized protocol applied at baseline.

Tissue Biomarkers

Research biopsy samples were shipped to Mayo Clinic Rochester for biomarker analyses. Tissue PGE₂ levels were assessed by a standardized method using EIA.¹² The intra-assay coefficient of variance is <10%, with a specificity of 100%, and a limit of detection 15 pg/mL. PGE₂ levels were corrected for tissue weight and represented as ng/gm of tissue. Samples were run in two batches, with the pre- and post-intervention biopsies obtained from a given participant run on the same plate.

Statistical Analyses

The primary endpoint was defined as absolute change in mean tissue PGE₂ concentration (post-intervention mean PGE₂ from up to 3 biopsy samples – baseline mean PGE₂ from up to 3 biopsy samples). Study participants were considered evaluable for the primary endpoint if they met eligibility criteria and PGE₂ data were available from both the pre- and post-intervention evaluations. The 1:1.5:1.5 randomization schema was based on the Pocock-Simon dynamic allocation procedure,¹⁴ which stratified by gender, length of Barrett's segment (< 5 cm versus ≥ 5 cm of circumferential involvement), baseline dysplasia status (none versus low-grade or indefinite), and participating site. Assuming equal standard deviations of 50 pg/mL for all 3 intervention arms, the sample sizes were determined to be n = 38, 57, and 57 in the aspirin placebo + PPI, lower dose aspirin + PPI, and higher dose aspirin + PPI arms, respectively (n = 152 total). These sample sizes yielded 80% power to detect a difference of at least 2/3 of a standard deviation in the mean PGE₂ concentration comparing each active aspirin arm vs. PPI alone, with a 2-sided alpha of 0.025 (adjusted for multiple comparisons). At least a 2/3 change in the standard deviation was selected as the minimum threshold for a clinically significant change because it was consistent with the goals for endpoint modulation of phase II chemoprevention trials, and this value represented the consensus goal defined by the investigative team in conjunction with the study sponsor (NCI Division of Cancer Prevention) to strike a reasonable balance between subject recruitment and data interpretation.

An unblinded pre-planned variability assessment (without halting accrual) in the first 40 participants was performed. This analysis showed slightly higher than expected variation in the PGE₂ values, where the standard deviation of the absolute change in PGE₂ values within

each arm varied from a low of 63 pg/mL to a high of 112 pg/mL. Despite the increased variability observed, we retained the original sample size due to the slower than anticipated accrual rate. In addition, due to concerns of slow accrual, we added interim futility and efficacy analyses so that the study could potentially close early at the time of the interim analyses. This interim analysis was planned after 77 participants were evaluable for PGE₂ (n = 19, 29, and 29 in the aspirin placebo + PPI, lower dose aspirin + PPI, and higher dose aspirin + PPI arms), during which each active aspirin arm was compared to the aspirin placebo arm using a 2-sample t-test (or nonparametric equivalent). Interim efficacy and futility boundaries were developed based on the O'Brien-Fleming method from the Lan-DeMets family¹⁵ using EAST software developed by Cytel.¹⁶ Each pair wise comparison decision was considered individually. If the 2-sided p-value was found to be < 0.0008 in favor of the active treatment arm(s), then significant efficacy would be declared. If the 2-sided p-value was > 0.634, then significant futility would be declared. The alpha and beta spent at the interim look were 0.001 and 0.036, respectively, and at the final analysis, a p-value < 0.0247 for either pair wise comparison in favor of the active treatment arm(s) would reject the null hypothesis and indicate that the active aspirin arm(s) were effective compared to the aspirin placebo arm, as per standard convention.¹⁵

Secondary endpoints included comparison of the percent change in PGE₂, baseline participant characteristics, agent adherence, and adverse events between intervention arms. Summary statistics and frequency tables were used to describe baseline subject characteristics, agent adherence, and adverse events. For participants that received more than the expected dose per protocol for any of the agents, we capped the percentage adherence numbers at 100%. Fisher's Exact and Kruskal-Wallis tests were used to test for associations between intervention arms and categorical and continuous data, respectively. Absolute and percent changes in PGE₂ were compared within each arm by the Wilcoxon signed rank procedure. The between-arm comparisons were carried out using the Wilcoxon rank-sum test. All statistical tests were performed 2-sided, unless otherwise noted using SAS version 9.2 (SAS Institute, Inc.).

RESULTS

Cohort Description

A CONSORT overview of participant recruitment is shown in Figure 1. One hundred eighty-one unique participants provided informed consent and were pre-registered for the baseline evaluation. A total of 59 participants were deemed screen failures. Reasons for screen failure included: inadequate Barrett's mucosa (n = 27), < 2 cm of circumferential BE (n = 20), participant or physician decision (n = 7), non-allowed concomitant medications (n = 2), and failure to meet other eligibility criteria (n = 3). Registered, ineligible participants, and intervention cohort participants were similar with respect to age, sex, smoking history, prior NSAID use, and lower dose aspirin use (p = 0.32) (Table 1).

Due to slower than anticipated accrual, randomization was closed early prior to reaching the *a priori* accrual goal of 152 participants. In total, 122 participants were randomized. Two participants were withdrawn post-randomization due to an inadequate number of surveillance biopsies and a finding of high-grade dysplasia at the pre-intervention endoscopy exam, respectively. With respect to race/ethnicity, 118/120 randomized participants (98%) were self-identified as Caucasian (race status was missing/unknown for 2 additional participants), while 109/120 (91%) and 6/120 (5%) were self-identified as non-Hispanic and Hispanic/Latino, respectively (ethnicity status was missing/unknown for 5 additional participants). Within the randomized cohort, 6 participants were not evaluable for the analyses of PGE₂ analyses due to sample-related issues (e.g., improper temperature, lost or delayed samples), leaving 114 evaluable participants for the primary endpoint.

Intervention Arms, Adverse Events and Agent Adherence

By design, intervention arms were evenly balanced with respect to sex ($p = 0.95$), length of BE ($p = 0.95$), and dysplasia status (no dysplasia vs. low grade dysplasia) at the pre-intervention evaluation ($p = 1.00$). Other baseline variables were similar between the intervention arms except for smoking history ($p = 0.005$) (Table 2).

Adverse events were reported in 26/120 (22%) trial participants after starting the assigned study intervention. Adverse event rates were similar across intervention arms (Table 2). Hiatal hernia erosions developed in one aspirin placebo participant and one lower dose aspirin participant. Antral erosions developed in one participant in the aspirin placebo arm. One higher dose aspirin participant experienced an erosion at the GE junction. No participants in any of the arms developed an ulcer. Agent adherence was excellent, with all participants receiving the majority of the assigned study doses, and was similar across the randomization arms ($p = 0.57$) (Table 2).

Tissue PGE₂ Concentration

The primary objective was to compare pre- and post-intervention biopsies within and between treatment arms based on PGE₂ concentrations in Barrett's mucosa. An interim analysis was performed after 77 participants were randomized ($n=19$, 29, and 29 in aspirin placebo, lower dose aspirin, and higher dose aspirin arms, respectively). The data were highly skewed, so the Wilcoxon Rank-Sum test was used to compare the active aspirin arms to the aspirin placebo arm. The 2-sided p -value was 0.0121 for the higher dose aspirin arm vs. aspirin placebo, which did not meet the interim analysis criteria to stop the trial for improved efficacy (2-sided p -value < 0.0008 required) or futility (2-sided p -value > 0.634 required). The 2-sided p -value was 0.0550 for the lower dose aspirin arm vs. aspirin placebo, which did not meet the interim analysis criteria to stop the trial for improved efficacy (2-sided p -value < 0.0008 required) or futility (2-sided p -value > 0.634 required). As such, the study continued on until it was decided to close early due to slow accrual.

Of the 120 participants in the intervention cohort, 114 were evaluable for the primary endpoint ($n = 29$, 42, and 43 in the aspirin placebo + PPI, lower dose aspirin + PPI, and higher dose aspirin + PPI arms, respectively). All evaluable participants had at least two PGE₂ values available from the pre- and post-intervention endoscopy exams (94% had three PGE₂ values). There was no statistically significant difference between the pre-intervention PGE₂ values across the three intervention arms ($p = 0.27$), but there was a statistically significant difference for the post-intervention PGE₂ values ($p < 0.0001$) (Table 3). Both the lower dose aspirin and higher dose aspirin arms showed significant decreases in PGE₂ from baseline for both absolute and percent change (Signed Rank $p = 0.0004$), while the aspirin placebo arm showed no significant change from baseline for absolute and percent change in PGE₂ values ($p = 0.27$). Based on the primary endpoint (absolute change in PGE₂), the higher dose aspirin + PPI intervention resulted in a significant decrease in PGE₂ values as compared to the aspirin placebo + PPI arm ($p = 0.02$) (Table 3 and Figure 2). Higher dose aspirin + PPI was also associated with a significant decrease in the percent change in PGE₂ values (secondary endpoint) compared to aspirin placebo + PPI ($p < 0.0001$) (Table 3 and Figure 3). Lower dose aspirin + PPI vs. aspirin placebo + PPI showed weaker results for absolute change ($p = 0.10$) (Table 3 and Figure 2), although there was still a trend toward a decrease in PGE₂ values. Based on percent change in PGE₂, lower dose aspirin + PPI showed a significant decrease in the PGE₂ values as compared to aspirin placebo + PPI ($p = 0.0007$) (Table 3 and Figure 3). No significant difference was observed between the two active aspirin arms for absolute ($p = 0.26$) or percent change ($p = 0.45$) in PGE₂ values. The primary endpoint analyses were not appreciably altered after excluding outliers (data not shown).

DISCUSSION

To our knowledge, we report the largest prospective chemoprevention trial in BE patients conducted in North America. Data from this clinical trial provide the first demonstration of a statistically significant effect from the combination of esomeprazole 40 mg bid and aspirin 325 mg qd for 28 days on tissue PGE₂ values in participants with BE, as compared to esomeprazole 40 mg bid without active aspirin. Although the lower dose aspirin (81mg qd) + PPI did not significantly alter tissue PGE₂ concentration, this finding was likely influenced by our relatively small sample size. Of note, a significant difference relative to the aspirin placebo + PPI group was observed when the percent decrease in PGE₂ levels was compared. These results support further evaluation of 325 mg aspirin administered together with twice daily esomeprazole as a potential chemoprevention strategy for BE patients in larger, more definitive phase III trials.

Esophageal adenocarcinoma is a lethal disease with a 5-year survival rate of approximately 17%.⁶ Survival is stage dependent and early spread prior to the onset of symptoms is characteristic of this tumor. Currently, the best hope for improved survival of patients with esophageal adenocarcinoma is detection of cancer at an early and potentially curable stage. In order to accomplish this, current strategies have focused on identifying more patients at risk, namely those with BE, in conjunction with widespread endoscopic surveillance of patients with known BE. Both strategies, however, are inherently flawed, expensive, and impractical. Furthermore, there are no prospective clinical data to confirm either approach is effective. Although observational studies have demonstrated surveillance to be associated with cancer downstaging with improved 5-year survival, the relevance of these studies is markedly limited by the risk of lead-time bias.^{17, 18} In this context, alternative intervention strategies, such as chemoprevention, are worthy of consideration, specifically during the early stages of neoplastic transformation.⁵

It is widely accepted that chronic injury and resulting inflammation play a major role in the development of several cancers, including esophageal adenocarcinoma.^{9, 19-23} The catalysis of arachidonic acid to PGE₂ by COX enzymes is thought to be critically involved in injury- or inflammation-induced esophageal carcinogenesis. Low pH, as well as exposure to caustic bile salts, can induce COX-2 expression in both ex-vivo culture models of BE and in esophageal adenocarcinoma cell lines.^{24, 25} COX-2 expression increases concomitantly with neoplastic progression in BE, thereby supporting the association of the arachidonic acid pathway with the development of esophageal adenocarcinoma.²⁶ Moreover, higher PGE₂ levels have been reported in Barrett's epithelium compared to normal squamous mucosa.²⁷ PGE₂ induces the proliferation of Barrett's epithelial cells, while PGE₂ inhibition reduces their proliferation.¹⁹ In addition, PGE₂ is known to diminish tumor surveillance by inhibiting natural killer cell activity.²⁸ Recent animal studies further demonstrate that inhibition of PGE₂ production with the use of selective and non-selective COX-2 inhibitors can decrease the rate of esophageal adenocarcinoma development.¹⁹ Thus, chronic induction of PGE₂ appears to stimulate esophageal carcinogenesis through multiple mechanisms.

A number of candidate chemoprevention agents have been proposed for BE patients including PPIs, selective and non-selective COX inhibitors (including aspirin), lyophilized black raspberries, antioxidants, green tea, retinoids, ursodeoxycholic acid, statins and curcumin.^{10, 29-31} Most attention has been directed toward the use of aspirin and other NSAIDs, which appear to lower esophageal cancer risk by favorably affecting COX-related pathways that are upregulated by excess exposure to acid and/or bile salts. While observational studies suggest that PPI therapy is associated with a decreased risk for the development of high-grade dysplasia and adenocarcinoma, the chemopreventive potential of PPIs, by suppressing acid and bile salt reflux, appears to be marginal when used as

monotherapy.³²⁻³⁴ There are a variety of possible explanations for this marginal effect, including the inability of PPIs to completely eliminate acid/bile salt reflux or activation of gastrin-cholecystekinin (CCK)-COX-2 mediated pro-carcinogenic signaling pathways. It is therefore logical to use a PPI agent in combination with NSAIDS, to determine if the combination more effectively targets intermediate markers of Barrett's-associated carcinogenesis.

Unfortunately, selective COX-2 inhibitors have been shown to increase the risk of thrombotic cardiovascular events making their widespread use for cancer chemoprevention problematic. An alternate approach is to use aspirin with a PPI, as was performed in our study. Aspirin decreases the risk of thrombotic cardiovascular events and is frequently recommended for patients at increased risk for myocardial infarction. In addition, PPIs may make aspirin a more useful and safer chemopreventive agent by minimizing aspirin-induced mucosal injury. Given the age-related increase in risk for gastrointestinal bleeding associated with NSAID use, this is a real concern for any chemoprevention strategy among BE patients, who tend to be of older age.³⁵ While PPIs, by targeting the root cause of the problem, may prevent chronic esophageal injury and decrease inflammation, aspirin use could control any COX-2 activation that may result from breakthroughs in acid suppression or gastrin activation by PPIs.

Clinical trial data regarding the potential chemopreventive effects of aspirin and other NSAIDS in BE patients are currently limited. One clinical trial examined the effect of celecoxib given for 48 weeks in participants with low-grade and high-grade dysplasia, and found no difference in the proportion of biopsy samples with dysplasia between participants treated with celecoxib compared to placebo.³⁶ Another small crossover study demonstrated that esomeprazole 40 mg twice daily in conjunction with aspirin at a dose of 325 mg daily administered for 10 days resulted in lower esophageal mucosal PGE₂ content in BE participants, whereas esomeprazole alone or in combination with rofecoxib did not reduce PGE₂ production.³⁴ To our knowledge, no additional clinical trial data have yet been reported on the potential effects of different doses of aspirin, in combination with PPI therapy, as a potential esophageal cancer chemopreventive strategy. The rationale for the use of the lower and higher dose of aspirin was to define if the smaller dose of aspirin was equally effective in decreasing tissue PGE₂ in Barrett's patients compared to the higher dose. Another reason to undertake such a dose ranging study is that the higher dose of aspirin in one colon cancer prevention study appeared to be less effective than the lower dose aspirin.³⁷

Strengths of the current study design included the avoidance of recruitment of participants with intestinal metaplasia of the cardia, the requirement for confirmation of intestinal metaplasia with goblet cells, and detailed interview to make sure patients were not unwittingly using aspirin or other NSAIDS prior to randomization. Thus, careful steps were taken to ensure a homogenous group of study participants with histologically-confirmed BE. Although our trial was conducted at multiple, geographically disparate sites, the relatively limited racial/ethnic diversity of our randomized cohort makes it unlikely that ethnopharmacology had any appreciable influence on our findings. While a significant difference was noted in the higher dose aspirin arm vs. the aspirin placebo arm, no such difference could be detected in the lower dose aspirin arm, despite a trend in that direction. The lack of a significant effect in the lower dose aspirin + esomeprazole arm may have been due to a lack of power and not a true lack of efficacy. The inability to achieve the *a priori* sample size, the lack of a sample size adjustment for the increased variability in PGE₂, and the use of planned non-parametric tests (instead of the parametric tests that the sample size was based on) all may have contributed to the study being under-powered to detect a significant effect for the lower dose aspirin + esomeprazole arm. As such, we cannot

conclude from our data that a combination of lower dose aspirin + esomeprazole does not provide chemopreventive benefits for BE patients.

There are a number of factors that make BE studies challenging to conduct. Importantly, many patients with BE already take aspirin or NSAIDs, which makes any such longer term clinical trial difficult to perform. Also, PGE₂ levels represent a surrogate end point for more direct clinical endpoints, such as progression to high-grade dysplasia and adenocarcinoma. There is no simple study design for assessing these more clinically relevant, yet substantially less common endpoints without considerable resource investment.

Our rationale to use PGE₂ as a surrogate biomarker of NSAID-related chemoprevention is based upon its direct role in carcinogenesis in Barrett's mucosa through upregulation of proliferation, resistance to apoptosis, and angiogenesis. Furthermore, PGE₂ biosynthesis is a downstream target of several oncogenic signals and it can be downregulated by tumor suppressors.³⁸⁻⁴⁰ The findings that carcinogenic bile salts in pH dependent manner upregulate PGE₂ biosynthesis along with increased expression of key regulators of PGE₂ by DNA damage and by aberrant p53 expression⁴¹ further support that the effect of genetic or epigenetic gains or losses that promote neoplasia could be inferred through a mechanistically relevant composite biochemical readout such as PGE₂ synthesis.

We believe that data from the current phase II trial provide strong support for a larger, more definitive phase III trial to further clarify the role of aspirin and esomeprazole in esophageal cancer chemoprevention among BE patients. Indeed, our study represents the largest chemoprevention trial in BE to date and has the advantage of examining a geographically diverse patient population. The ongoing multi-center phase III AsPECT trial, which is being conducted in the United Kingdom and elsewhere in Europe, is also examining aspirin and esomeprazole as a combination chemopreventive intervention for BE patients,⁴² with results that should serve to complement these findings.

In conclusion, data from this multi-center, prospective phase II trial demonstrate that twice daily esomeprazole in combination with higher dose (325 mg) daily aspirin therapy can favorably affect tissue PGE₂ levels in BE patients. Although the lower dose aspirin (81 mg) was not found to significantly reduce PGE₂ levels as compared to esomeprazole alone based on the primary endpoint analysis, data from our secondary analyses suggest that esomeprazole twice per day in combination with 81 mg aspirin per day may also provide some chemopreventive benefits for BE patients. As such, a larger trial is recommended to more fully investigate the aspirin dose effect to see what aspirin dose is optimal. Given the importance of PGE₂ and related molecular pathways in Barrett's-associated carcinogenesis, this dual regimen of aspirin and esomeprazole warrants further evaluation as a novel chemoprevention strategy for an increasingly common disease with potentially devastating clinical outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. CONSORT Diagram that shows the number of participants that were pre-registered, the number of screen failures, along with the number of participants that were randomized and evaluable for the primary endpoint.

Figure 2.

Boxplots for the Absolute Change in PGE₂ by Intervention Arm. After adjusting for the interim analysis and multiple comparisons, the nominal significance level was 0.0247. As shown, the Higher Dose Aspirin + PPI intervention resulted in a significant decrease in PGE₂ values as compared to Aspirin Placebo + PPI ($p = 0.020$), while the Lower Dose Aspirin + PPI intervention did not differ significantly from Aspirin Placebo + PPI ($p=0.096$).

Figure 3.

Boxplots for the Percent Change in PGE₂ by Intervention Arm. As shown, the Higher Dose Aspirin + PPI and Lower Dose Aspirin + PPI interventions resulted in significant decreases in PGE₂ values as compared to Aspirin Placebo + PPI ($p < 0.001$).

Table 1
Baseline demographics by trial participation status

	All Registered (N=181)	Ineligible or Withdrawn (N=61)	Intervention Cohort (N=120)	p-value
Age in Years				0.80 ¹
Mean (SD)	59.9 (10.9)	60.4 (10.2)	59.7 (11.2)	
Median	60	61	60	
Range	32-86	35-84	32-86	
Sex, N (%)				1.00 ²
Female	28 (15.5)	9 (14.8)	19 (15.8)	
Male	153 (84.5)	52 (85.3)	101 (84.2)	
Smoking History [*] , N (%)				0.32 ²
Current Smoker	22 (12.2)	10 (16.7)	12 (10.0)	
Never Smoked	80 (44.4)	23 (38.3)	57 (47.5)	
Quit/Former Smoker	78 (43.3)	27 (45.0)	51 (42.5)	
Prior History of Chronic NSAID Use? [*] , N (%)				0.62 ²
No	160 (88.9)	52 (86.7)	108 (90.0)	
Yes	20 (11.1)	8 (13.3)	12 (10.0)	
Prior History of Lower Dose Aspirin Use? [*] , N (%)				0.47 ²
No	135 (75.0)	43 (71.7)	92 (76.7)	
Yes	45 (25.0)	17 (28.3)	28 (23.3)	

¹Kruskal Wallis

²Fisher Exact

^{*} missing data from 1 participant, who had unknown values (this participant was excluded from the p-value calculation).

Table 2
Baseline demographics, intervention adherence, and adverse events by intervention arm

	Arm A [aspirin placebo + PPI] (N=30)	Arm B [lower dose aspirin + PPI] (N=45)	Arm C [higher dose aspirin + PPI] (N=45)	Overall (N=120)	p- value
Age					0.93 ¹
Mean (SD)	60.1 (11.3)	59.7 (11.7)	59.3 (11.0)	59.7 (11.2)	
Median	61	59	59	60	
Range	(32-86)	(34-81)	(35-82)	(32-86)	
Sex, N (%)					0.95 ²
Male	26 (86.7)	37 (82.2)	38 (84.4)	101 (84.2)	
Female	4 (13.3)	8 (17.8)	7 (15.6)	19 (15.8)	
ECOG Performance Score, N (%)					1.00 ²
0	29 (96.7)	44 (97.8)	44 (97.8)	117 (97.5)	
1	1 (3.3)	1 (2.2)	1 (2.2)	3 (2.5)	
Length of the Barrett's Segment, N (%)					0.95 ²
<5 cm if circumferential involvement	15 (50.0)	21 (46.7)	21 (46.7)	57 (47.5)	
>=5cm of circumferential involvement	15 (50.0)	24 (53.3)	24 (53.3)	63 (52.5)	
Dysplasia Status at Pre-Intervention, N (%)					1.00 ²
no dysplasia	26 (86.7)	38 (84.4)	38 (84.4)	102 (85.0)	
low grade or indefinite for dysplasia	4 (13.3)	7 (15.6)	7 (15.6)	18 (15.0)	
Smoking History, N (%)					0.005 ²
Current Smoker	3 (10.0)	3 (6.7)	6 (13.3)	12 (10.0)	
Never Smoked	9 (30.0)	31 (68.9)	17 (37.8)	57 (47.5)	
Quit/Former Smoker	18 (60.0)	11 (24.4)	22 (48.9)	51 (42.5)	
Prior History of Chronic NSAID Use?, N (%)					0.40 ²
No	29 (96.7)	40 (88.9)	39 (86.7)	108 (90.0)	
Yes	1 (3.3)	5 (11.1)	6 (13.3)	12 (10.0)	
Prior History of Lower Dose Aspirin					1.00 ²

	Arm A [aspirin placebo + PPI] (N=30)	Arm B [lower dose aspirin + PPI] (N=45)	Arm C [higher dose aspirin + PPI] (N=45)	Overall (N=120)	p- value
Use², N (%)					
No	23 (76.7)	35 (77.8)	34 (75.6)	92 (76.7)	
Yes	7 (23.3)	10 (22.2)	11 (24.4)	28 (23.3)	
Intervention Adherence					
Esomeprazole					
Median (Range) # of pills taken	56 (37-64)	54 (44-69)	56 (37-75)	55 (37-75)	0.63 ¹
% Adherence ^a , Median (Range)	100 (71-100)	100 (79-100)	100 (71-100)	100 (71-100)	0.57 ¹
Mean (SD)	98 (5.5)	99 (4.7)	98 (5.5)	98 (5.2)	
Aspirin/Placebo 81mg					
Median (Range) # of pills taken	28 (25-32)	27 (23-35)	28 (17-38)	28 (17-38)	0.48 ¹
% Adherence ^a , Median (Range)	100 (84-100)	100 (84-100)	100 (61-100)	100 (61-100)	0.99 ¹
Mean (SD)	99 (3.0)	99 (2.7)	99 (6.0)	99 (4.3)	
Aspirin/Placebo 325mg					
Median (Range) # of pills taken	28 (25-32)	27 (23-35)	28 (23-38)	28 (23-38)	0.61 ¹
% Compliance ^a , Median (Range)	100 (96-100)	100 (84-100)	100 (93-100)	100 (84-100)	0.79 ¹
Mean (SD)	100 (0.9)	99 (2.6)	99 (1.6)	99 (2.0)	
Treatment-Emergent Adverse Events					
Any Adverse Event Regardless of	5 (16.7)	8 (17.8)	13 (28.9)	26 (21.7)	0.36 ²
Treatment Relation or Grade					
Adverse Events Related to Study Medication	5 (16.7)	6 (13.3)	3 (6.7)	14 (11.7)	0.39 ²
Grade 1 Adverse Events	6 (20.0)	7 (15.6)	10 (22.2)	23 (19.2)	0.77 ²
Grade 2 Adverse Events	2 (6.7)	1 (2.2)	5 (11.1)	8 (6.7)	0.27 ²
Grade 3 Adverse Events	0 (0.0)	1 (2.2)	1 (2.2)	2 (1.7)	1.00 ²

¹ Kruskal Wallis

² Fisher Exact, %compliance=100*[(total # pills taken)/[(# days pills taken)*(scheduled #pills/day)]]

Table 3
PGE2 Summary in all 114 evaluable participants

PGE2 Measurement	Arm A [aspirin placebo + PPI] (N=29)	Arm B [lower dose aspirin + PPI] (N=42)	Arm C [higher dose aspirin + PPI] (N=43)
Pre-Evaluation *			
Mean (SD)	210.9 (244.18)	178.3 (249.4)	204.2 (265.53)
Median (Range)	149.9 (12.5 to 1024.6)	63.4 (2.0 to 1019.1)	110.8 (2.4 to 1140.2)
Post-Evaluation *			
Mean (SD)	143.3 (129.51)	54.3 (148.9)	29.3 (35.33)
Median (Range)	92.3 (12.9 to 518.0)	13.6 (1.5 to 916.7)	17.0 (1.6 to 182.3)
Absolute Change			
Mean (SD)	-67.6 (229.68)	-123.9 (284.0)	-174.9 (263.62)
Median (Range)	0.0 (-745.2 to 454.7)	-47.3 (-976.6 to 789.0)	-93.1 (-1113.6 to 75.3)
Within Arm	0.27	<0.0001	<0.0001
Signed Rank p-value			
Wilcoxon Rank-Sum p-value **		vs. aspirin placebo p = 0.0955 ** lower dose vs. higher dose aspirin p = 0.26	vs. aspirin placebo p = 0.0204 **
Percent Change			
Mean (SD)	45.0 (183.03)	-24.5 (143.1)	-58.9 (63.05)
Median (Range)	0.0 (-86.8 to 717.8)	-77.6 (-98.2 to 618.1)	-83.6 (-98.7 to 242.1)
Within Arm Signed Rank p-value	0.68	0.0004	<0.0001
	–	vs. aspirin placebo	vs. aspirin placebo
Wilcoxon Rank-Sum p-value		p = 0.0007 lower dose vs. higher dose aspirin p = 0.45	p < 0.0001

* PGE2 values were similar at the pre-evaluation (P=0.27) and significantly different at the post-evaluation (P<0.0001).

** the nominal significance level was 0.0247 after adjusting for the interim analysis and multiple comparisons.