Aspirin Dose

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Background: A variety of studies have supported the finding that regular intake of aspirin (acetylsalicylic acid) or nonsteroidal anti-inflammatory agents can affect colorectal cancer carcinogenesis. These agents inhibit the synthesis of prostaglandins. High levels of prostaglandins are observed in colon cancer tissues. Purpose: Experiments were planned to determine the lowest dose of aspirin that can markedly suppress the levels of mucosal prostaglandins E2 and F2α in colorectal mucosa and to determine whether a relationship exists between these levels and plasma levels of both acetylsalicylic acid and its metabolite, salicylic acid. Methods: Healthy men and women aged 18 years or older participated in the study. The participants took a single, daily dose of aspirin (40.5, 81, 162, 324, or 648 mg) or a placebo for 14 days. Colorectal biopsy specimens were taken at baseline, 24 hours after the first dose of aspirin, and 24-30 hours and 72-78 hours after the last dose. The suppression occurred without detectable amounts of aspirin or salicylic acid in the plasma at the time points studied. On the basis of these observations, we recommend a single, daily dose of 81 mg of aspirin in future studies of this drug as a chemopreventive agent for colorectal cancer.

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See "Notes" following "References."
Many investigations have focused attention on the hypothesis that nonsteroidal anti-inflammatory drugs, including aspirin, could reduce the incidence of or mortality from colorectal cancer. Several lines of evidence obtained from animal studies, tumor and cell culture studies, human tissue studies, and human studies support this hypothesis. Numerous animal studies (1-12) have demonstrated that nonsteroidal anti-inflammatory drugs inhibit colon tumor development in rodents previously exposed to chemical carcinogens. Tumor and cell culture work (13,14) has demonstrated that acetylsalicylic acid (aspirin) and other nonsteroidal anti-inflammatory drugs alter the cycle and proliferation of colon cancer cells. Human tissue studies (15,16) have found higher levels of prostaglandin E2 in colon cancer samples than in paired histologically normal colon samples. The preclinical data consistently support the theory that acetylsalicylic acid or nonsteroidal anti-inflammatory drugs inhibit carcinogenesis in the colon.

The human studies exploring the relationship between colorectal cancer and the use of aspirin and other nonsteroidal anti-inflammatory drugs have been observational or interventional. Nearly all of the observational studies (17-28) found a significant reduction in colorectal cancer risk for those patients regularly taking aspirin or nonsteroidal anti-inflammatory drugs. In contrast, a single prospective cohort of elderly California residents (29) found no protective effect of nonsteroidal anti-inflammatory drugs. Three large clinical trials of aspirin for prevention of large-bowel adenomas or colorectal cancer (23,27,30) have produced conflicting results. Greenberg et al. (23) demonstrated a lower risk of adenomas at 1-year follow-up among patients with resected adenomas who took aspirin regularly. In contrast, Gann et al. (30) found no effect of low-dose aspirin on colorectal cancer among participants in the Physicians’ Health Study after a mean follow-up of 5 years. A similar study among women in the Nurses’ Health Study (27) found a substantial reduction in the risk of colorectal cancer, but not until after at least a decade of aspirin use. There is some uncertainty with regard to the relationship between use of aspirin or other nonsteroidal anti-inflammatory drugs and colorectal cancer, but most studies on humans still demonstrate some favorable outcome.

The mechanism by which nonsteroidal anti-inflammatory drugs may reduce cancer mortality is unknown (31-37), but these agents are known to inhibit cyclooxygenase and to reduce prostaglandin synthesis. The tissue concentration of prostaglandin E2 has been shown to be significantly increased in colorectal adenomas, malignant colonic polyps, and gross cancer compared with normal flat mucosa (38). High levels of prostaglandin E2 have been found in local venous blood draining colon carcinomas and in the peripheral blood in patients with liver or lung metastases (39). Recent evidence (40-42) suggests that cyclooxygenase-2 plays an important role in cellular growth.

Because of these preliminary data, we have embarked upon studies to develop aspirin as a chemopreventive agent for human colorectal cancer. Our first goal was to define the relationship of aspirin dose, pharmacokinetics, and prostaglandin concentration in human colorectal mucosa to select a chemopreventive dose and schedule for aspirin.

Subjects and Methods

Subjects

Healthy human subjects were recruited to participate in a study of the effect of aspirin on prostaglandin concentration in colorectal mucosa. This study involved the taking of blood and colorectal biopsy specimens without preparation of the rectum with laxatives or enemas. To be eligible for the study, the participants were required to be 18 years of age or older, to be able to give written informed consent, and to be in normal health, as demonstrated by medical history, physical examination, and normal hematochemical status (i.e., white blood cell count >4000/mm³, hemoglobin level >12 g/dL, platelet count >120,000/mm³, and prothrombin time and partial thromboplastin time in control range). Participants were excluded if they were pregnant or lactating, were suffering from other concomitant medical illnesses, were taking medications within 1 week of the study, or were unwilling to stop taking aspirin or nonsteroidal anti-inflammatory drugs. All participants were reimbursed for their time. The protocol was reviewed and approved by the University of Michigan Human Use Committee. A total of 65 subjects (10 receiving placebo, groups of 10 each receiving 40.5, 81, 162, or 324 mg aspirin, and a group of 15 receiving 648 mg of aspirin) completed the protocol.

Diet

Beginning 2 days before biopsy specimens were obtained, the dietary intake of the participants was controlled to reduce potentially confounding variables. Specifically, a meal plan was developed to distribute energy and macronutrients equally in each of three meals per day, so that variable meal size and composition would not interfere with variations attributable to diurnal variations. Meal plans were individualized to accommodate different energy requirements for weight maintenance among the subjects; these energy requirements ranged from 1800 to 3000 kcal/day (7531-12,552 kJ/day). All subjects were required to eliminate caffeine-containing beverages from their diet.

Toxicity Assessment

Subjects were assessed for toxic effects by direct questioning at frequent intervals (4, 8, and 24 hours after the first dose, again at 7 and 14 days during daily dosing, and after the final biopsy specimens were taken). Symptoms germane to aspirin toxicity were solicited by the research coordinator. The National Cancer Institute (NCI) Common Toxicity Criteria scale (Regulatory Affairs Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, Diagnosis, and Centers, NCI, Bethesda, MD) and the Costa Toxicity Frequency Scale (43) were used to quantify toxicity. Subjects experiencing Common Toxicity Criteria grade 2 or grade 1 and Costa Toxicity Frequency Scale 2 were considered to have unacceptable toxic effects and were removed from the study.

Plasma Collection

All subjects were admitted to the Clinical Research Center prior to their first dose. A blood specimen was obtained at baseline, and the first drug dose was taken. Blood specimens were collected at 10, 20, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after the first dose. The specimens were collected in heparinized tubes on ice with 100 μL of a 5000/g solution of KF-2H₂O added to acidify the plasma and to stabilize the acetylsalicylic acid and salicylic acid present in the plasma. The blood was immediately centrifuged at 1200g for 10 minutes at 4°C. Plasma was removed on ice and stored in a plastic tube (Falcon, Fisher Scientific, Pittsburgh, PA) at −70°C until assay.

Assay of Plasma for Acetylsalicylic Acid and Salicylic Acid

Acetylsalicylic acid (aspirin) and its metabolite salicylic acid were assayed from 1 mL of plasma placed in a glass screw cap tube and spiked with 10 μL of internal standard (m-hydroxybenzoic acid, 70 mg/L). To this solution were added 1 mL of 1.0 M oxalic acid and 10 mL of hexane–ether (1:1 volume). The tubes were capped and shaken vigorously for 1 minute and then centrifuged at 514g for 5 minutes at 4°C (GPR refrigerated centrifuge; Beckman Instruments, Inc., Fullerton, CA). The organic layer was transferred to a clean glass conical screw-cap centrifuge tube, and 300 μL of 0.5 M phosphate buffer (pH 7.0) was added. The tubes were capped, shaken vigorously for 1 minute, and then centrifuged at 514g for 5 minutes at 4°C. We transferred 200 μL of the aqueous phase to a clean test tube. Then 200 μL of 1.0 M phosphate buffer (pH 2.0) was added and vortex mixed for 10 seconds. The sample was transferred to a low-volume insert for high-performance liquid chromatography (HPLC) assay. HPLC conditions were as follows: mobile
phase, 0.2 M phosphate buffer (pH 2.5)—HPLC-grade water—acetonitrile (4:3:3); flow rate, 1 mL/minute; column, Spherisorb ODS-2.5 micron (Alltech, Deerfield, IL); detector, Waters model 484 UV detector (Waters Corp., Milford, IL) set at 232 nm; pump, Waters model 600E Controller; and injector, Waters model 700 WISP auto injector (44,45).

Tissue Collection
At the time of the colorectal biopsy, the participants were placed in a left lateral decubitus position. A flexible sigmoidoscope was passed to 10-15 cm from the anal sphincter. Eight tissue samples were taken by opening and pressing the biopsy forceps perpendicular to the mucosal surface with mild pressure. Each biopsy specimen was taken far enough away from other biopsy sites to avoid sampling an area affected by the previous biopsies. The initial protocol required taking colorectal biopsy specimens at baseline, 2 hours after the first dose, 24 hours after the first dose, and 24-30 hours after the final dose. This protocol was followed for the first 15 subjects taking 648 mg of aspirin daily. Subsequent participants had biopsies in a similar manner at baseline, 24 hours after the first dose, and 24-30 hours and 72-78 hours after the last daily dose.

Tissue Analysis
Each biopsy specimen (approximate weight, 5 mg) was placed immediately into a sterile 1.6-mL Eppendorf tube and frozen in liquid nitrogen within 20 seconds. The specimens were stored at −70°C until immediately before analysis. A single colorectal biopsy specimen was removed from the freezer, placed in 0.5 mL of phosphate-buffered saline at room temperature, immediately homogenized for 30 seconds, vortex mixed for precisely 2 minutes, and then immediately centrifuged at 2100 g for 30 seconds at room temperature. The supernatant was removed, and two 50-μL aliquots were assayed for prostaglandin E2 and prostaglandin F2α with the use of a competitive enzyme immunoassay kit from Cayman Chemical Co. (Ann Arbor). If the prostaglandin value determined did not fit within the limits of the assay, then another 50 μL of the supernatant was diluted 1:10 or 1:100 with phosphate-buffered saline and again assayed for prostaglandins. This process was repeated until the prostaglandin value measured was within the limits of the assay. We assayed 6 μL of the supernatant for crude protein by using a Coomassie Microtiter Plate (Pierce Chemical Co., Rockford, IL) protein assay. The prostaglandin concentration (in picograms) was normalized per microgram protein. The prostaglandin assays were performed in 10 batches. All biopsy samples from a given individual were assayed in the same batch to eliminate any batch effect in within-subject responses.

Drug
A single lot of 81-mg tablets of baby aspirin with dextrose as the carrier was provided as Bayer’s Children’s Formulation (Bayer Corporation, Consumer Care Division, Morristown, NJ) as a single, recently manufactured lot (lot No. KC3326). The drug was given as a single, daily dose of one half, one, two, four, or eight tablets (40.5 mg, 81 mg, 162 mg, 324 mg, or 648 mg, respectively). The pills were swallowed, not chewed. The control subjects took a placebo containing dextrose. Subjects were not randomly assigned to dose groups, but they were assigned in the following order to one of the dose groups: 648 mg, 40.5 mg, 324 mg, 81 mg, 162 mg, and placebo. Equal numbers of men and women were assigned to each drug dose level. Adherence was monitored by self-report and electronic monitoring of pill consumption (Medication Event Monitoring; Apex Corp., Fremont, CA) (46). Subjects were classified as being adherent if the self-report and electronic monitoring suggested that 80% or more of the doses were taken as prescribed. Subject’s data were not used if either method of monitoring adherence noted extra doses of aspirin taken during the 14-day course or after aspirin dosing was to be stopped.

Analysis
Acetylsalicylic acid and salicylic acid plasma concentrations were calculated and stored by use of Excel 5.0 (Microsoft Corp., Redmond, WA). For curve fitting and calculation of the area under the plasma–time–concentration curve (AUC), the time–concentration files were uploaded into Kaleidagraph (Synergy Software, Reading, PA). In Kaleidagraph, the time–concentration curves were graphically displayed, and the elimination portion of the time–concentration curves was fit to a two-compartment, exponential elimination function with parameters estimated by use of least-squares criteria and the AUC calculated as follows:

\[ C_t = Ae^{-kt} + Be^{-kt'}, \]

where \( C_t \) is the concentration of aspirin at any given time \( t \), \( A \) is the intercept on the y axis at time zero for the distribution phase, \( B \) is the intercept on the y axis at time zero for the elimination phase, \( e \) is the natural log exponent, and \( x \) axis represents time in hours. The distribution rate constant \( k_d \) and the elimination rate constant \( k_e \) were obtained from the slope of the best fit of the distribution and elimination phases of both aspirin and salicylic acid time–concentration curves.

Other pharmacokinetic parameters were calculated by use of noncompartmental methods described by Rowland and Tozer (47). The elimination half-life (\( t_{1/2} \)) was calculated from the following equation:

\[ t_{1/2} = 0.693k_e. \]

The total-body clearance (\( CL_{TB} \)) for aspirin was calculated by the following equation:

\[ CL_{TB} = D_o/AUC. \]

where \( D_o \) is the total dose administered. The AUC from time zero to infinity was used. The volume of distribution (\( V_d \)) was calculated as follows:

\[ V_d = D_o/C_{in}. \]

where \( C_{in} = A + B \) and \( A \) and \( B \) are obtained from the two-compartment, exponential elimination fit. \( C_{max} \) was estimated from the time–concentration curve as the maximum concentration point after absorption for aspirin or after metabolism for salicylic acid. \( T_{max} \) was defined as the time after drug administration at which \( C_{max} \) occurred.

The tissue prostaglandin and plasma data were merged with the demographic data to make a single data file. All statistical analyses were performed by use of the SAS System (SAS Institute Inc., Cary, NC).

The prostaglandin measurements were performed in 10 batches. All biopsy specimens from a given subject were assayed in the same batch to eliminate any batch effect in within-subject responses. Analysis of variance was used to compare the values at each measurement point between batches. Because we observed that the standard deviations of prostaglandin concentrations increased alongside the means in various subgroup analyses and we expected aspirin to decrease prostaglandin concentrations in individual subjects proportionally from their baseline values, we logarithmically transformed (to base 10) prostaglandin concentrations and the ratios of concentrations at a given time to the baseline values. To maintain between-subject independence of measurement, all ratios were calculated within subjects and any statistics concerning ratios were calculated on within-subject ratios.

The primary outcome of interest was the effect of aspirin on colorectal mucosal prostaglandins E2 and F2α. We anticipated that the effect of aspirin would be a decrease in colorectal prostaglandin E2 and prostaglandin F2α concentrations concordant with dose. We used Williams’ test (48,49) to evaluate the differences in prostaglandin E2 and prostaglandin F2α concentrations across the various dose levels. Williams’ test is appropriate in situations where the expected effect is monotonically increasing or decreasing with dose; like Dunnett’s test, Williams’ test compares drug-treated groups with a placebo-treated group, but it is more powerful by construction than Dunnett’s test against ordered alternative hypotheses. Williams’ test is also useful for determining the lowest effective dose, since once an overall effect has been established. In Williams’ test, three steps are required. First, a standard analysis of variance was used to estimate the within-group standard deviation. The different aspirin dose group means were displayed with the means of the placebo group as the referent group. Second, the dose groups were combined until the within-group means were ordered. Third, a test of overall treatment effect was performed. In this test, r-statistics calculated from these ordered means were compared with tabulated values that were somewhat larger than the percentiles of the standard Student’s t distribution. If the test of an overall treatment effect was statistically significant, the lowest effective dose was determined. The test for the lowest effective dose was adjusted by Bonferroni’s method to protect against multiple comparison effects. All P values are two-sided.

Results
Study Population
Sixty-six subjects were initially enrolled in the study. The subjects consisted of 34 (52%) men and 32 (49%) women. Their mean age was 27.8 years (range, 19-56 years). One male subject was removed from the study before completing the protocol because of drug-induced toxic effects. The following results are from the remaining 65 subjects, except when adherence did not meet criteria (two
subjects in the 40.5-mg group took all doses improperly; moreover, the following subjects took an extra dose on day 15: two subjects in the 40.5-mg group, one subject in the 81-mg group, two subjects in the 162-mg group, and one subject in the 324-mg group). Several prostaglandin assay data points were eliminated because the assay procedure for these individual samples could not produce results within the linear portion of the standard curve and tissue specimens were not available to repeat assays not fitting the curve (one subject in the 162-mg group at all assay points, 14 subjects at baseline, and three subjects for assay at 2 hours after the first dose, 24 hours after the first dose, and 24-30 hours after the last dose on day 14 in the 648-mg dose group).

Toxicity

Of the 66 subjects studied, one subject reported unacceptable drug-induced toxic effects. This subject developed persistent dark-red rectal bleeding during the once-daily aspirin dosing of 648 mg. Despite requests to inform research staff of any persistent rectal bleeding, the subject did not report the rectal bleeding and light-headedness until 7 days after the biopsy performed at 24 hours after the first dose of aspirin. Rigid sigmoidoscopy revealed persistent bleeding from the biopsy sites.

The bleeding was controlled by cautere
tation, and aspirin was discontinued. Follow
ing the cauterization, the subject continued to have colorectal pain without bleeding. Subsequent evaluations found an anal fissure not amenable to medical therapy. Surgical repair resolved the problem. The rectal bleeding was deemed related to both the aspirin and the biopsy procedures.

Six other subjects had tolerable drug-induced toxic effects. Two subjects had minimal gastric discomfort reported only on day 14 of aspirin intake; both were taking an aspirin dose of 162 mg. Another subject taking a daily dose of 324 mg of aspirin reported indigestion on days 9-14 that resolved by day 17. One subject taking a daily dose of 324 mg of aspirin reported mild fatigue on days 14 and 17. Two subjects reported episodes of headache. One subject taking a daily dose of 81 mg of aspirin reported periodic headaches on day 14 that were resolved by day 17. Another subject taking a daily dose of 81 mg of aspirin reported mild headaches on day 14 that were resolved by day 17. Six subjects taking aspirin at doses of 162, 324, 40.5, 40.5, 40.5, and 81 mg reported less than grade 1 rectal bleeding (NCI Common Toxicity Criteria scale) following the rectal biopsies. The remaining subjects did not report any quantifiable toxic effects.

Table 1. Pharmacokinetic parameters of acetylsalicylic acid (aspirin) and salicylic acid after a single dose*

<table>
<thead>
<tr>
<th>Acetylsalicylic acid dose, mg</th>
<th>No. of subjects</th>
<th>Mean BMI,† kg/m²</th>
<th>AUC,‡ (µg/mL) × h</th>
<th>Cmax,§ µg/mL</th>
<th>Tmax,¶ h</th>
<th>Vₐ,≠ L</th>
<th>Cl₁₅/₁₈ ** L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Aspirin</td>
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<tr>
<td>648</td>
<td>14</td>
<td>24.3</td>
<td>6.56 ± 4.44</td>
<td>0.50 ± 0.23</td>
<td>5.16 ± 3.21</td>
<td>0.72 ± 0.38</td>
<td>93.9 ± 46.5</td>
</tr>
<tr>
<td>324</td>
<td>9</td>
<td>22.9</td>
<td>2.87 ± 1.38</td>
<td>0.38 ± 0.08</td>
<td>3.76 ± 2.00</td>
<td>0.45 ± 0.22</td>
<td>55.3 ± 17.7</td>
</tr>
<tr>
<td>162</td>
<td>10</td>
<td>25.4</td>
<td>1.92 ± 0.77</td>
<td>0.40 ± 0.19</td>
<td>2.17 ± 1.08</td>
<td>0.55 ± 0.21</td>
<td>71.4 ± 58.5</td>
</tr>
<tr>
<td>81</td>
<td>10</td>
<td>23.9</td>
<td>0.98 ± 0.54</td>
<td>0.60 ± 0.40</td>
<td>1.13 ± 1.00</td>
<td>0.53 ± 0.23</td>
<td>106.1 ± 86.0</td>
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<tr>
<td>40.5</td>
<td>10</td>
<td>22.9</td>
<td>0.67 ± 0.85</td>
<td>0.41 ± 0.40</td>
<td>0.68 ± 0.38</td>
<td>0.47 ± 0.21</td>
<td>49.6 ± 31.3</td>
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<tr>
<td>B. Salicylic acid</td>
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<tr>
<td>648</td>
<td>14</td>
<td>24.3</td>
<td>243.94 ± 64.3</td>
<td>3.10 ± 0.83</td>
<td>37.24 ± 12.41</td>
<td>1.98 ± 0.84</td>
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<td>324</td>
<td>9</td>
<td>22.9</td>
<td>149.15 ± 59.1</td>
<td>2.88 ± 0.89</td>
<td>25.68 ± 5.87</td>
<td>1.12 ± 0.43</td>
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<tr>
<td>162</td>
<td>10</td>
<td>25.4</td>
<td>42.76 ± 14.51</td>
<td>2.46 ± 1.20</td>
<td>10.94 ± 3.16</td>
<td>1.25 ± 0.45</td>
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</tr>
<tr>
<td>81</td>
<td>10</td>
<td>23.9</td>
<td>16.34 ± 8.61</td>
<td>1.61 ± 0.83</td>
<td>4.76 ± 1.96</td>
<td>1.43 ± 0.73</td>
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<tr>
<td>40.5</td>
<td>10</td>
<td>22.9</td>
<td>5.44 ± 4.42</td>
<td>3.34 ± 3.04</td>
<td>1.63 ± 1.13</td>
<td>0.93 ± 0.40</td>
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</tr>
</tbody>
</table>

*The total number of subjects for this aspect of our study was 53. Ten subjects taking placebo did not complete the pharmacokinetic data collection; two subjects (one taking 648 mg and one taking 324 mg) had incomplete pharmacokinetic data.
†Body mass index.
‡Area under the curve from time zero extrapolated to infinity.
§Peak measured concentration.
¶Time to peak measured concentration.
≠Volume of distribution of the terminal excretion phase. Not measured for the metabolite salicylic acid.
**Total-body clearance. Not measured for the metabolite salicylic acid.

Acetylsalicylic Acid and Salicylic Acid Concentrations in Plasma

The acetylsalicylic acid half-life ranged from 0.38 to 0.60 hour (Table 1, A). The salicylic acid half-life ranged from 1.61 to 3.34 hours (Table 1, B). Both were consistent across doses. Compared with acetylsalicylic acid, salicylic acid had a half-life that was sixfold longer, an AUC that was eightfold to 52-fold higher, and maximal concentrations that were twofold to sevenfold higher. There was no detectable acetylsalicylic acid or salicylic acid in plasma 24-30 hours after a single dose or after multiple daily doses for 2 weeks.

Colorectal Mucosal Prostaglandins

The mean baseline concentration of colorectal mucosal prostaglandin E₂ in biopsy specimens was 25.8 pg/µg protein (range, 2.7-125.7 pg/µg protein) (Fig. 1). The mean baseline concentration of colorectal mucosal prostaglandin F₂α in biopsy specimens was 12.1 pg/µg protein (range, 0.3-161.3 pg/µg protein) (Fig. 2). There was significant variation in the measurements of prostaglandin E₂ (P<0.0001) and prostaglandin F₂α (P<0.0001) between batches. Batch effects accounted for 51% of the prostaglandin E₂ variation and 49% of the prostaglandin F₂α variation at baseline. The differences...
between men and women were not significant for colorectal mucosal prostaglandin E₂ (P = .97) and prostaglandin F₂α (P = .80), adjusted for assay batch.

The ratio of prostaglandin E₂ (Fig. 3, A) and prostaglandin F₂α (Fig. 3, B) to the respective baselines generally decreased with increasing dose, but the relationship was not consistently monotonic. Williams’ test was used on the ratios to establish an overall drug effect and to select the lowest effective dose. The lowest dose to significantly suppress colorectal mucosal prostaglandin E₂ from baseline at 24 hours after the first dose and 24-30 hours after the last dose was 162 mg (suppression by 22.6% and by 14.2%; P = .002 and .021, respectively). At 72-78 hours after the last dose, the lowest dose to significantly suppress colorectal mucosal prostaglandin E₂ was 81 mg (suppression by 23.7%; P = .008). The lowest dose to significantly suppress colorectal mucosal prostaglandin F₂α from baseline at 24 hours after the first dose was 324 mg (suppression by 18.3%; P = .032). The lowest aspirin dose required at 24-30 hours and 72-78 hours after the last dose was 40.5 mg (15.1% and 23.0% suppression; P = .003 and .0002, respectively).

Discussion

Our data suggest that a single, low dose of aspirin taken once daily inhibits colorectal mucosal prostaglandin synthe-
sis. While batch-to-batch assay variations caused some difficulties in the analysis of the data, overall we found that an aspirin dose of 81 mg taken daily is sufficient to significantly reduce colorectal mucosal prostaglandins. There was a difference between the aspirin doses sufficient to suppress colorectal mucosal concentrations of prostaglandin E₂ and prostaglandin F₂α. The lowest dose of aspirin required to suppress prostaglandin E₂ concentration approximately 24-30 hours after 14 daily doses was 162 mg; in contrast, at 72-78 hours after discontinuation of aspirin dosing, only 81 mg was required to suppress mucosal prostaglandin E₂ concentration. We are unable to completely resolve this discrepancy, but we attribute this difference to assay technology specifically associated with the prostaglandin E₂ assay or to the large variation in prostaglandin E₂ concentration within individuals. The data we obtained for prostaglandin F₂α were more uniform and demonstrated that a lower aspirin dose, 40.5 mg, was sufficient to significantly suppress this prostaglandin. Given these differences, we believe that daily oral dosing with 81 mg of aspirin will deliver sufficient drug to colorectal mucosa to significantly suppress prostaglandin synthesis.

The plasma pharmacokinetics of acetylsalicylic acid and salicylic acid (Table 1) measured in the study subjects were consistent with similar pharmacokinetic
data reported in the literature (50). The mean half-lives of aspirin and salicylic acid and time to maximal concentrations predict no measurable aspirin or salicylic acid in the plasma 12 hours after an oral dose. No aspirin concentrations in any of our subjects were detected at time points of 8 hours or later after a single dose or at any of the times measured 24-30 hours or 72-78 hours after the final dose of 14 daily doses. Salicylic acid was detected in the plasma from 17 of 53 subjects at baseline prior to aspirin intake. Eight of these subjects continued to have detectable amounts of salicylic acid 24-30 hours after the last dose on day 14. Another five subjects had no detectable amounts of salicylic acid in the plasma at baseline, but they were noted to have detectable amounts of salicylic acid 24-30 hours after the last dose on day 14. Low salicylic acid concentrations prior to aspirin treatment and at 12 hours or 24-30 hours after treatment are possibly diet induced (51).

Salicylic acid is present in substantial concentrations in commonly consumed foods such as fruits (especially berry fruits and dried fruits) and in teas, herbs, and spices (52), but it appears to have low bioavailability (51). The discrepancy is noteworthy between aspirin and salicylic acid plasma pharmacokinetics and pharmacodynamic effects as measured by colorectal mucosal prostaglandin suppression. While such durable suppression has been observed in the human platelet, thus leading to recommendations of daily or longer dosing intervals for cardiovascular protection (53-56), the human platelet is non-nucleated and incapable of regenerating cyclooxygenases. Colorectal epithelial and mucosal cells are nucleated, yet our data suggest that they are incapable of regenerating active cyclooxygenases in vivo after aspirin-induced acetylation. The recently published data of DuBois et al. (57) support the concept that aspirin’s prolonged pharmacodynamic activity may be due to its transcriptional inhibition of the cyclooxygenase-2 gene. Prolonged binding to a negative transcriptional regulatory element might explain these pharmacodynamic effects.
This study has some limitations to be considered before one should promote the use of colorectal mucosal prostaglandins as a surrogate endpoint biomarker for colorectal cancer. First, our study population consisted entirely of healthy, young adults. The impact of aspirin on colorectal mucosal prostaglandins may be different in older adults or in patients with significant risk of colorectal cancer (e.g., family history of adenomatous polyps or colorectal cancer). Second, colorectal mucosal prostaglandins have not been linked to risk of developing colorectal cancer. One of the key criteria to be accepted as a surrogate endpoint biomarker is that it be associated with specific stages of cellular or molecular events associated with multistep carcinogenesis (58). Third, we encountered technical difficulties in using the competitive enzyme immunosorbent assay kit for assaying the colorectal mucosal prostaglandin concentrations. As shown, there was significant variation in colorectal mucosal prostaglandin concentrations according to batch assay. The basis of this variation remains unclear. However, this problem does not reduce the validity of the findings because we compared each subject’s response to his or her baseline value and all individual subject measurements were assayed in the same batch.

The concentrations of colorectal mucosal prostaglandin E2 have been published by Finley et al. (59) using a radioimmunoassay and bicinchoninic acid assay for protein determination in biopsy specimens. The concentrations ranged from 0.13 pg to 0.36 pg prostaglandin E2/μg of protein. The concentrations reported in this study at baseline were significantly larger. Finley et al. (59) had much larger biopsy specimens (12.0-22.0 mg), used a different assay technique, exposed all subjects to tap water enemas before the biopsy, studied a different subject population with regard to risk of colorectal cancer, and processed the tissue with the instillation of indomethacin buffer immediately after biopsy. Comparisons of the prostaglandin values reported here and those reported by Finley et al. (59) are extremely difficult and may not be valid.

On the basis of the data presented here, we believe that additional prospective trials of aspirin as a chemopreventive agent for colorectal cancer are warranted. While we are aware of at least two large, randomized, prospective chemoprevention trials on the use of aspirin (60) as a polyph preventive agent, phase IIA chemoprevention trials on aspirin and other nonsteroidal anti-inflammatory drugs are warranted to determine whether these agents will modulate other potential surrogates of colorectal carcinogenesis prior to a definitive risk reduction trial in a large subject cohort.

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Notes
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