Antagonistic Effects of Aspirin and Folic Acid on Inflammation Markers and Subsequent Risk of Recurrent Colorectal Adenomas

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The Aspirin/Folate Polyp Prevention Trial found that aspirin, but not folic acid, reduced recurrence of colorectal adenomas. This study examined whether treatment effects on inflammation markers explained the trial results. The trial had a factorial design with three aspirin (placebo, 81, and 325 mg/d) and two folic acid (placebo and 1 mg/d) groups. There were 884 subjects who had colonoscopic evaluation for adenomas at year 3 and plasma levels of C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), soluble TNF receptor type II (sTNF-R2), and IL-1 receptor antagonist (IL-1Ra) measured at baseline and year 3. Among individuals not receiving folic acid, there was a 4% decrease (mean ratio of year 3 to baseline levels = 0.96, 95% confidence interval [CI] = 0.82 to 1.14) in CRP for a period of 3 years in the 325 mg of aspirin group vs a 20% increase (mean ratio = 1.20, 95% CI = 1.03 to 1.41) in the placebo group (P = .027). By contrast, the reverse was observed among individuals who also received folic acid (Pinteraction = .013). Changes in inflammation markers were not associated with adenoma recurrence. Low-dose aspirin (325 mg/d) is beneficial in stabilizing CRP levels, which may be abrogated by folate. Nevertheless, inflammation markers do not mediate the chemopreventive effect of aspirin on colorectal adenomas.


Both observational studies and clinical trials have demonstrated consistently the efficacy of low-dose aspirin (≤325 mg/d) in the chemoprevention of colorectal adenomas (1–4), but the antineoplastic mechanisms of low-dose aspirin are not known. Because inflammation is thought to be involved in the etiology of colorectal neoplasia (5–7), we hypothesized that low-dose aspirin could change the circulating levels of inflammatory cytokines and thereby reduce risk of colorectal neoplasms.

We conducted secondary analyses in the Aspirin/Folate Polyp Prevention Study (2,8), a clinical trial of aspirin and/or folic acid for the prevention of recurrence of colorectal adenomas, to examine several hypotheses. First, we evaluated the effects of daily low-dose aspirin and folic acid on circulating levels of proinflammatory markers, namely C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), and on anti-inflammatory cytokine receptors, including soluble TNF receptor type II (sTNF-R2) and IL-1 receptor antagonist (IL-1Ra). Although IL-6 and TNF-α may have growth-promoting and oncogenic bioactivities as indicated in laboratory studies (9–11), the soluble receptors sTNF-R2 and IL-1Ra block binding of TNF and IL-1, respectively, to their cell-surface receptors, resulting in potent inhibition of the activities of these proinflammatory cytokines (12,13). Therefore, our second objective was to assess whether changes in these inflammation markers affected risk of recurrent adenomas. Finally, we examined if the previously observed treatment effects of aspirin and folic acid on adenoma recurrence were mediated by these inflammation markers.

In the double-blind randomized Aspirin/Folate Polyp Prevention Study (2,8), patients with a recent history of colorectal adenomas were recruited between July 6, 1994, and March 20, 1998, into the trial with a 3 × 2 factorial design consisting of three aspirin groups (placebo, 81, and 325 mg/d) and two folic acid groups (placebo and 1 mg/d) (clinicaltrials.gov identifier: NCT00272324). At recruitment, patients completed a questionnaire regarding their personal characteristics, medical history, and lifestyle habits. Adenoma recurrence was determined by colonoscopy and pathology review 3 years later, and at that time, aspirin treatment was terminated. Participants were invited to continue their blinded folic acid assignment until their next colonoscopy, which was performed about 3–5 years after the year 3 examination. All patients provided written informed consent, and institutional review board approval was obtained at each participating clinical center. The trial found that subjects treated with 81 mg of aspirin, but not 325 mg of aspirin, had statistically significantly reduced risk for recurrent adenomas (2). By contrast, folic acid supplementation was associated with an increased risk of advanced lesions (tubulovillous, villous, or large adenomas; high-grade dysplasia; or invasive cancer) (8).

The present analysis examined data from randomization through the year 3...
colonoscopy; it included 884 participants who had nonfasting blood samples collected at both baseline and year 3 visits (Figure 1). These 884 participants were similar to those who were excluded from the analysis with respect to demographic and medical characteristics. Their paired plasma samples, which were stored at −70°C, were assayed simultaneously for inflammation markers by the following methods: CRP by latex-enhanced immunonephelometry (interassay correlation of variation = 4%; Behring Diagnostics, San Jose, CA), IL-6 by an ultrasensitive solid-phase enzyme-linked immunosorbent assay (interassay correlation of variation = 14%; R&D Systems, Minneapolis, MN), TNF-α and IL-1Ra together by a multiplex assay (interassay correlation of variations of 13% and 13%, respectively; Human Cytokine/Chemokine panel; Millipore, Billerica, MA), and sTNF-R2 by another multiplex assay (interassay correlation of variation = 9%; Death Receptor Kit; BioSource International, Camarillo, CA). To determine the effects of aspirin and folic acid treatments on inflammation markers, levels of inflammation markers were first natural log transformed to improve normality and change in an inflammation marker (δ) was calculated as [ln (year 3 level) − ln (baseline level)]. In linear regression analysis, δ of each inflammation marker (dependent variable) was regressed against the main effects of aspirin (3 groups) and folic acid (2 groups) randomizations as well as the interaction between aspirin and folic acid with adjustment for covariates that were associated with that particular inflammation marker. The adjusted mean of δ for each treatment group was obtained from the regression model, and the geometric mean of δ was equivalent to the mean ratio of year 3 to baseline marker levels. To examine whether changes in inflammation markers were associated with risk of recurrent adenomas, the two outcome variables, any adenoma and advanced adenomas at year 3, were analyzed by log-linked binomial regression models. Change in each inflammation marker was analyzed as an independent variable with adjustment for inflammation marker level at baseline and established risk factors for colorectal adenomas. To evaluate whether changes in inflammation markers might mediate the effects of aspirin and folic acid treatments on recurrence of adenomas, we examined relative risks for the associations of aspirin and folic acid treatments with recurrence of adenomas while controlling for established adenoma risk factors from two log-linked binomial regression models: 1) with change in inflammation marker (ie, the potential mediator) entered into the model and 2) without such an adjustment. Attenuated relative risks from the former model, as compared with the latter, would suggest that treatment effects might be partially mediated by the inflammation marker that was entered in the model. All statistical tests were two-sided, and differences for which P values were less than .05 were considered statistically significant.

The average age of patients was 57 (±9) years, 86% were whites, and 64% were males. There were no important differences in baseline characteristics, including the levels of inflammation markers, among the six randomization groups. Among individuals who did not receive folic acid, those who took 325 mg of aspirin had stable levels of proinflammatory markers, particularly CRP, for a period of 3 years (Figure 2). There was a negligible 4% decrease (mean ratio of year 3 to baseline levels = 0.96, 95% confidence interval [CI] = 0.82 to 1.14) in CRP levels at year 3 in the 325 mg of aspirin group as compared with a 20% increase (mean ratio = 1.20, 95% CI = 1.03 to 1.41) in the placebo group (P = .027 comparing the two groups). However, among individuals who also received folic acid, the reverse trend was observed. For example, TNF-α levels at year 3 increased by 39% (mean ratio = 1.39, 95% CI = 1.09 to 1.77) among subjects taking 325 mg of aspirin, whereas the placebo group had virtually no change (mean ratio = 0.98, 95% CI = 0.76 to 1.26) in their TNF-α levels (P = .023 comparing the two groups). The antagonistic interaction between folic acid and 325 mg of aspirin was statistically significant for CRP (P = .013) and TNF-α (P = .038) and was not for IL-6 (P = .063). Folic acid given alone as well as 81 mg of aspirin, given with or without folic acid, had no effect on plasma levels of CRP, TNF-α, and IL-6. Treatments had no effect on the levels of sTNF-R2 or IL-1Ra (data not shown).

Changes in plasma levels of CRP, TNF-α, and IL-6 were further assessed for their clinical significance; none was associated with risk of adenoma recurrence (Table 1). Hence, it became unlikely that these markers of inflammation could mediate the effects of aspirin and folic acid. Indeed, when the relative risks for associations of treatment groups with adenoma risk after accounting for change in CRP, TNF-α, or IL-6 were compared with those without such an adjustment, the former relative risks were attenuated by less than 5%, suggesting that treatment effects were not explained by these inflammation markers. As for sTNF-R2 or IL-1Ra, neither their baseline levels nor 3-year changes were related to recurrent adenomas (data not shown).
It is known that a high dose of aspirin (>3 g/d) is required to achieve its clinical anti-inflammatory effects (14). However, it is unclear whether low-dose aspirin (81–325 mg/d) has a modest anti-inflammatory effect, particularly on lowering the levels of proinflammatory cytokines, because previous clinical trials had small sample sizes and treatment periods of less than 2 months (15–18). Our study demonstrated that in the absence of folic acid treatment, long-term intake of 325 mg of aspirin daily precluded CRP plasma concentrations from escalating over time. However, subjects who did not receive any treatment (double-placebo) had elevated levels for a 3-year period. It has also been shown in the general population that CRP increases with age (19). Our data suggest that 325 mg of aspirin stabilizes the levels of CRP. Higher doses may be necessary to further reduce the levels of proinflammatory markers.

In our study, the levels of inflammation markers for a period of 3 years among subjects who received 1 mg of folic acid alone were not statistically significantly different from those in the double-placebo group. Our data and the majority of previous studies (20–25) do not support an important anti-inflammatory role of folate in modulating inflammatory cytokines.

The antagonistic interaction between 325 mg of aspirin and folic acid, such that the two regimens together increase the levels of proinflammatory markers, needs to be confirmed in future studies. The biological mechanism for such an interaction is unknown. A few studies (26–28) have reported that high doses of aspirin have antifolate effects, which may be crucial for the anti-inflammatory action. If so, one can speculate that low-dose aspirin in the presence of high folic acid levels from supplementation may not yield the antifolate activity needed to have an effect on inflammatory cytokines. Nevertheless, our study highlights the potential complexity of combining the two regimens for primary or secondary disease prevention.

Changes in levels of CRP, IL-6, and TNF-α in the 3-year period had trivial clinical significance. These changes were not associated with the risk of recurrent adenomas nor did they mediate the effects of aspirin and folic acid on risk of adenomas. It has been shown that inflammatory responses and cytokine signaling in intestinal epithelial cells may be responsible for the pathogenesis of chronic intestinal disorders (29). Therefore, the lack of associations of recurrent adenoma risk with proinflammatory and anti-inflammatory...

Figure 1. Design of the trial and flow of participants.

Figure 2. Adjusted mean ratios of year 3 to baseline inflammation marker levels by randomization group among 884 subjects in this study. A) CRP = C-reactive protein. B) TNF-α = tumor necrosis factor α. C) IL-6 = interleukin 6. Without folic acid supplementation (squares). With 1 mg of folic acid (triangles). With 1 mg of folic acid and 1 mg of folic acid alone (diamonds). With 1 mg of folic acid supplementation (circles).
markers in our study could be because of the fact that plasma instead of tissue levels of inflammation markers were measured. It is also possible that inflammation markers are associated with the initial development of colorectal adenomas but not with recurrence.

In summary, our data suggest that low-dose aspirin has modest effects on stabilizing CRP, which may be abrogated by a high level of folate. However, such beneficial effects do not appear to confer protection against colorectal neoplasia. Inflammation markers do not mediate the previously observed effects of aspirin and folic acid on colorectal adenomas.

References


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