Folic Acid and Risk of Prostate Cancer: Results From a Randomized Clinical Trial

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Data regarding the association between folate status and risk of prostate cancer are sparse and conflicting. We studied prostate cancer occurrence in the Aspirin/Folate Polyp Prevention Study, a placebo-controlled randomized trial of aspirin and folic acid supplementation for the chemoprevention of colorectal adenomas conducted between July 6, 1994, and December 31, 2006. Participants were followed for up to 10.8 (median = 7.0, interquartile range = 6.0–7.8) years and asked periodically to report all illnesses and hospitalizations. Aspirin alone had no statistically significant effect on prostate cancer incidence, but there were marked differences according to folic acid treatment. Among the 643 men who were randomly assigned to placebo or supplementation with folic acid, the estimated probability of being diagnosed with prostate cancer over a 10-year period was 9.7% (95% confidence interval [CI] = 6.5% to 14.5%) in the folic acid group and 3.3% (95% CI = 1.7% to 6.4%) in the placebo group (age-adjusted hazard ratio = 2.63, 95% CI = 1.23 to 5.65, Wald test *P* = .01). In contrast, baseline dietary folate intake and plasma folate in nonmultivitamin users were inversely associated with risk of prostate cancer, although these associations did not attain statistical significance in adjusted analyses. These findings highlight the potential complex role of folate in prostate cancer and the possibly different effects of folic acid–containing supplements vs natural sources of folate.

J Natl Cancer Inst 2009;101:432–435

Folate is a major carrier of one-carbon groups needed for methylation reactions and nucleotide synthesis (1). Although there is currently concern about a possible harmful role of folate in colorectal cancer (2,3), its effects on prostate cancer are not clear (4–12).

We report here secondary findings regarding prostate cancer incidence in the context of a double-blind randomized clinical trial of aspirin and/or folic acid for the prevention of colorectal adenomas, the Aspirin/Folate Polyp Prevention Study (13). Patients eligible for the trial had a recent history of colorectal adenomas and no contraindication, or need, for aspirin or folate. A study colonoscopy was scheduled 3 years after the qualifying examination to assess adenoma recurrence. Subsequently, subjects were invited to remain in study follow-up and to continue the folic acid treatment to which they had been randomly assigned until the time of an additional colonoscopy, which was usually performed 3–5 years after the year 3 examination. Informed consent was obtained for each participant, and institutional review boards at each institution approved the research.

At study entry, all participants completed a risk factor questionnaire and a semiquantitative food frequency questionnaire (14); nonfasting blood samples were collected and used to measure circulating levels of folate and other B vitamins. Quality control procedures and assay coefficients of variation (CVs) have been reported elsewhere (15–17). For plasma vitamin B_6, within-day CV and between-day CVs were 5.1%–7.4%; for riboflavin the range was 3.1%–7.8% (15).

While on study treatment, subjects were sent questionnaires every 4 months regarding adherence to treatment, use of medications and supplements, and medical events. Similar questionnaires were sent annually to subjects while they were under observational follow-up. Medical records were obtained for all patient reports of prostate cancer, and the diagnosis was confirmed by histopathology.

Of the 712 men originally randomly assigned, four had a history of prostate cancer, 61 had been randomly assigned to aspirin only, and four did not complete any study questionnaire, leaving 643 men for this analysis (Figure 1). Spearman rank correlation coefficient was used to calculate the correlation between dietary (excluding supplemental) and circulating levels of folate (Spearman rho correlation coefficient = 0.25, $P < .001$). We used Kaplan–Meier curves and Cox proportional hazard models to assess the association between folic acid supplementation and prostate cancer incidence. Graphical evaluation by Schoenfeld residual plots indicated that the model assumptions concerning proportional hazards were appropriate (18). We used $1 - S(t)$ to estimate absolute risks of prostate cancer over defined follow-up periods, where $S(t)$ is the Kaplan–Meier probability of surviving free of prostate cancer. All analyses of effects of folic acid treatment were conducted according to the principle of intention to treat. To analyze dietary intake, we computed the residuals of the regression of the logarithm of the dietary folate on the logarithm of calories and used the logarithm of caloric intake to adjust for energy intake (19). Circulating

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See "Funding" and "Notes" following "References."

DOI: 10.1093/jnci/djp019

levels and dietary intake of folate were modeled as continuous variables adjusted for potential confounders (age; alcohol use; log of caloric intake [dietary only]; baseline multivitamin use; dietary intake or plasma levels of vitamins B_2, B_6, and B_{12}; aspirin treatment group; folic acid treatment group) as indicated in Table 1. All statistical tests were two-sided.

The mean age of men included in the secondary analysis was 57.4 (SD = 9.2) years; 41 (6.4%) of the 643 men were African American. Of the 643 men, 595 (93%) agreed to follow-up beyond 3 years and 472 (73%) continued the assigned folic acid (or placebo) treatment. The characteristics of the men in the two treatment groups were similar. We found no statistically significant differences between placebo and folic acid treatment groups for dietary folate (placebo [mean] = 350, 95% CI = 330 to 370 mg/d); folic acid = 337, 95% CI = 320 to 354 mg/d), plasma folate (placebo = 22.7, 95% CI = 20.7 to 24.7 nmol/L; folic acid = 23.2, 95% CI = 21.1 to 25.2 nmol/L), and red blood cell folate (placebo = 404.1, 95% CI = 388.2 to 420.0 nmol/L; folic acid = 388.6, 95% CI = 373.6 to 403.6 nmol/L). However, those randomly assigned to folic acid supplementation had statistically significantly lower baseline plasma vitamin B_{12} (placebo = 348.1 pmol/L; folic acid = 317.0 pmol/L, difference = 31.1 pmol/L, 95% CI = 1.8 to 60.4 pmol/L, $P = .04$).

A total of 34 subjects were diagnosed with prostate cancer after random assignment. Detailed information regarding clinical stage was available for only 17 of them, but only three cancers were known to have spread beyond the capsule. The mean Gleason score (available for all patients) was 6.4 (SD = 1.1) and did not differ between treatment groups. There was no statistically significant effect of aspirin on risk of prostate cancer, but there was a marked increase in risk in subjects randomly assigned to folic acid relative to those in the placebo group (Figure 2, Table 1). The estimated probability of being diagnosed with prostate cancer in the folic acid group was 9.7% (95% CI = 6.5% to 14.5%) over 10 years and in the placebo group it was 3.3% (95% CI = 1.7% to 6.4%). The age-adjusted hazard ratio (HR) was 2.63 (95% CI = 1.23 to 5.65, $P = .01$).

In contrast to the direct association of folic acid supplementation with risk of prostate cancer in the randomized analysis, there were possible indications of inverse associations of prostate cancer risk with dietary folate intake (multiadjusted HR = 0.65, 95% CI = 0.35 to 1.20), and with baseline plasma folate among subjects who did not use multivitamins (multiadjusted HR = 0.42, 95% CI = 0.17 to 1.04, Table 1). Dietary intake of B_2 and B_6 and plasma B_2, B_6, B_{12} was not associated with risk (data not shown).

The reason for the contrasting associations of folate supplementation and baseline intake or circulating levels of folate with the risk of prostate cancer is unknown. Folate supplementation in the trial was in the form of folic acid, a fully oxidized, monoglutamyl form of folate that may differ in its effects from the natural reduced and methylated forms (mostly 5-methyl tetrahydrofolate). Folic acid is more bioavailable than natural sources (20); natural folates exist as polyglutamates that are hydrolyzed to monoglutamates in the small intestine and then polyglutamated in peripheral cells (21). The borderline inverse association of dietary intake and plasma levels (among nonmultivitamin users) is consistent with some observational data that natural folates could be protective against prostate cancer (7). However, protective dietary associations could be confounded by other factors if foods rich in folate also contain protective nutrients or are associated with protective lifestyle habits.

CONTEXT AND CAVEATS

Prior knowledge

Some observational studies had suggested that increased folate in the diet might lower the risk of prostate cancer.

Study design

This study addressed the effect of folic acid supplementation on risk of prostate cancer in the context of a double-blind randomized clinical trial of folic acid and/or aspirin for prevention of colorectal adenoma. Dietary intake of folate was assessed at baseline.

Contribution

Folic acid supplementation was associated with increased risk of prostate cancer. By contrast, baseline dietary folate was inversely associated with prostate cancer risk.

Implications

Folate metabolism may have a complex role in prostate cancer; the effects of folic acid–containing supplements on the risk of prostate cancer may be different those of natural dietary sources.

Limitations

Given the small number of prostate cancers in this study, the estimates of prostate cancer risk in the placebo and folic acid groups should be interpreted with caution.

From the Editors

There are plausible biological reasons why high levels of folate may promote carcinogenesis. Folates have a key role in the synthesis of nucleotides (1,2) that are

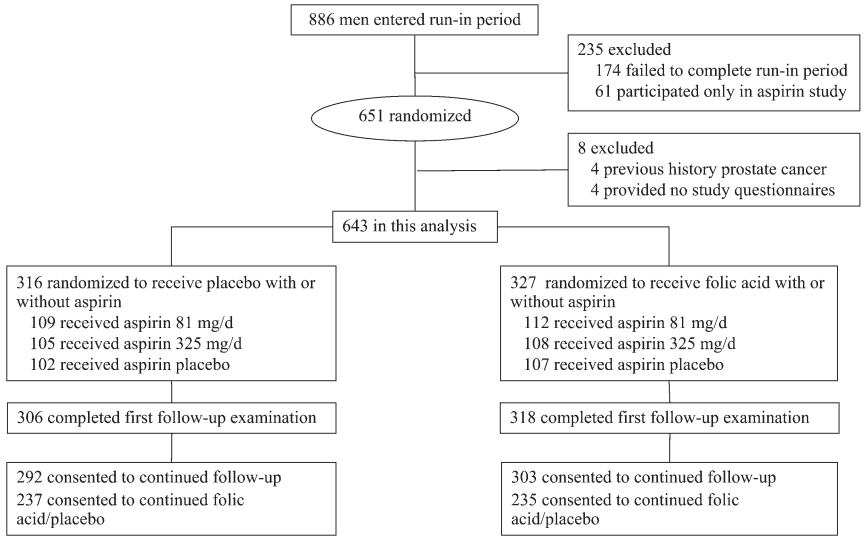

Figure 1. Design of the trial and flow of participants.

Table 1. Association of folic acid treatment, baseline dietary intake, and circulating levels of folate with risk of subsequent prostate cancer*

	Adjusted for age		Adjusted for multiple factors	
Risk factor	**HR (95% CI)†**	***P* value†**	**HR (95% CI)**	***P* value**
Folic acid supplementation	2.63 (1.23 to 5.65)	.01	2.58 (1.14 to 5.86)‡	.02
Dietary folate§	0.70 (0.48 to 1.04)	.08	0.65 (0.35 to 1.20)‖	.17
Nonmultivitamin users	0.77 (0.50 to 1.19)	.24	0.70 (0.37 to 1.33)‖	.28
Multivitamin users	0.53 (0.21 to 1.32)	.17	0.49 (0.18 to 1.36)‖	.17
Plasma folate¶	0.56 (0.32 to 0.98)	.04	0.68 (0.35 to 1.30)#	.25
Nonmultivitamin users	0.41 (0.18 to 0.96)	.04	0.42 (0.17 to 1.04)#	.06
Multivitamin users	1.01 (0.55 to 1.86)	.97	1.11 (0.58 to 2.15)#	.75
Red blood cell folate**	0.82 (0.57 to 1.17)	.27	1.19 (0.77 to 1.83)#	.43
Nonmultivitamin users	0.73 (0.46 to 1.16)	.18	0.91 (0.54 to 1.53)#	.64
Multivitamin users	1.81 (0.95 to 3.45)	.07	2.04 (1.08 to 3.87)#	.03

* Cox proportional hazard models were used to obtain hazard ratios and 95% confidence intervals. HR = hazard ratio; CI = 95% confidence interval.

† Adjusted for age. For dietary folate, also adjusted by baseline total caloric intake.

‡ Adjusted for age; aspirin treatment group; alcohol use; baseline multivitamin use; and plasma levels of vitamins B_2, B_6, and B_{12}.

§ HR per one SD of the residual of the log-log–transformed dietary folate (SD = 0.4) using methods described in the text.

‖ Adjusted for age; alcohol use; aspirin treatment group; baseline multivitamin use; dietary intake (foods only) of calories, B_2, and B_6; and folic acid treatment group.

¶ HR per one SD of the level of plasma folate (SD = 18 nmol/L).

Adjusted for age; alcohol use; aspirin treatment group; baseline multivitamin use; plasma levels of B_2, B_6, and B_{12}; and folic acid treatment group.

** HR per one SD of the level of red blood cell folate (SD = 142 nmol/L).

needed for proliferating neoplastic cells, and there is an increase in the expression of folate receptors in several cancer types (22). Prostate-specific membrane antigen, a transmembrane-carboxypeptidase with folate hydrolase activity (23,24), is overexpressed in nearly all prostate cancers, and higher tissue levels of this protein are associated with higher grade, higher Gleason score (25), and disease recurrence (26). Folate also has a key role in DNA methylation (1,2), and alterations in methylation patterns including global DNA hypomethylation (27) and gene-specific hypomethylation (28) appear to be important events in prostate cancer. Unmetabolized folic acid has recently been associated with reduced natural killer cell cytotoxicity (29), which could impair first-line defense against malignant cells (30).

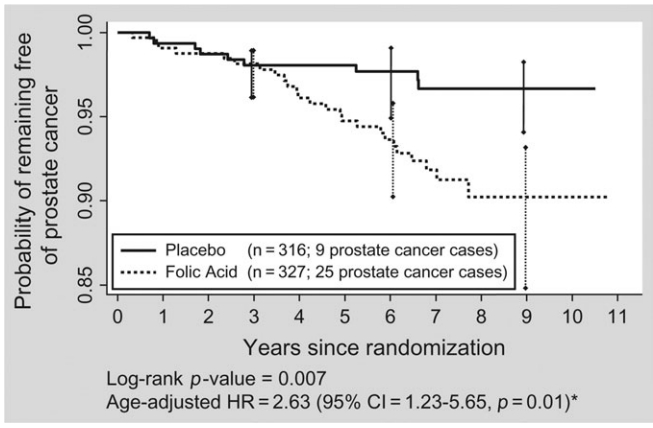

Figure 2. Kaplan–Meier plot of the prostate cancer–free status over time among the 643 men randomized to placebo and folic acid treatment in this study. The 95% confidence intervals (CIs) for probability of remaining free of cancer are shown at 3, 6, and 9 years for both treatment groups. The numbers of patients at risk for prostate cancer at 3, 6, and 9 years were 284, 274, and 10, respectively, in the placebo group and 300, 233, and 10 in the folic acid group. *Cox proportional hazard models were used to obtain hazard ratios (HRs) and 95% confidence intervals and Wald tests to obtain *P* values. In the figure, hazard ratio is age adjusted. The multiadjusted hazard ratio (with adjustment for aspirin treatment group, alcohol use, baseline multivitamin use and plasma levels of vitamins B_2, B_6, and B_{12}) was 2.58 (95% CI = 1.14–5.86, $P = 0.02$).

Our study has several limitations. It was a secondary analysis, and the estimates of risk may not be precise given the small number of prostate cancers observed. No systematic attempts were made to detect early cancers through a protocol-driven rectal examination or measurement of prostate-specific antigen. Therefore, we could not control for screening. In addition, food frequency questionnaires may be subject to error. Lastly, most of the subjects in our cohort who developed prostate cancer had localized tumors with an average Gleason score of 6, so we could not investigate effects on advanced prostate cancer.

Strengths of this study include the random assignment of folic acid supplementation, which minimized the possibility of confounding, and its prospective design, which minimized recall biases. Medical records were obtained for all subjects who were reported to have developed prostate cancer, with a blinded review to verify diagnosis and ascertain disease characteristics.

In conclusion, this clinical trial provides evidence that daily supplementation with 1 mg of folic acid was associated with an increased risk of prostate cancer. It is unclear why dietary and plasma levels among nonmultivitamin users may be inversely associated with risk. These findings highlight the potentially complex role of folate in prostate carcinogenesis.

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Funding

Supported in part by grants (R01-CA-059005 and U54-CA-100971) from the National Cancer Institute, National Institutes of Health; supported in part by a post-PhD Research Fellowship from the National Cancer Institute of Canada (#017602 to J.C.F.).

Notes

We thank all the individuals who participated in this clinical trial. Wyeth, which markets folate supplements, provided study agents for the clinical trial that this research is based on. R. S. Bresalier is a consultant for Prozen, Inc. The study sponsors had no role in the study design, data collection, analysis and interpretation of the data, the decision to submit the manuscript for publication, or the writing of the manuscript (clinicaltrials.gov Identifier: NCT00272324).

Manuscript received July 2, 2008; revised December 11, 2008; accepted January 16, 2009.