Does Folic Acid Decrease Plasma Homocysteine and Improve Endothelial Function in Patients With Predialysis Renal Failure?

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Background—Considerable evidence suggests that hyperhomocysteinemia is an independent vascular risk factor that promotes atherosclerosis by inducing endothelial dysfunction. Although folic acid reduces hyperhomocysteinemia, the effect on adverse vascular events is unknown. We hypothesized that in patients with chronic renal failure, a condition associated with both hyperhomocysteinemia and atherosclerosis, treatment with folic acid would improve endothelial function.

Methods and Results—In a prospective, double-blind protocol, 100 patients (mean age 62 years, 67 men) with predialysis chronic renal failure were randomized to 5 mg folic acid or placebo daily for 12 weeks. Endothelial function was assessed by measuring (1) endothelium-dependent dilation of the brachial artery, (2) combined serum nitrite/nitrate concentrations, and (3) plasma von Willebrand factor concentration. Baseline characteristics of the 2 groups were similar. At the end of the study, both serum and red cell folate concentrations were greater in the folic acid group than the placebo group [mean (95% CI) 39.0 (29.8 to 51.0) versus 7.7 (6.6 to 8.9) μg/L and 739 (613 to 891) versus 220 (184 to 262) μg/L, respectively; both $P<0.001$]. Despite a reduction in hyperhomocysteinemia in the folic acid group compared with the placebo group [15.1 (14.1 to 16.2) versus 20.1 (18.2 to 22.2) μmol/L; $P<0.001$], there were no significant differences in endothelium-dependent dilation, combined serum nitrite/nitrate concentrations, or plasma von Willebrand factor concentration between the 2 groups.

Conclusions—High-dose folic acid lowers but fails to normalize hyperhomocysteinemia in patients with predialysis chronic renal failure. This was not accompanied by an improvement of endothelial function and suggests that treatment with folic acid may not reduce the burden of vascular disease in uremia. **(*Circulation*. 2000;102:871-875.)**

Key Words: atherosclerosis ■ endothelium ■ kidney ■ amino acids

Epidemiological evidence indicates that a raised plasma concentration of homocysteine, a sulfur-containing amino acid, is an independent risk factor for the development of atherosclerotic disease.[1] Renal function is a major determinant of plasma homocysteine concentration, and patients with chronic renal failure have severe hyperhomocysteinemia.[2] Cardiovascular disease is the leading cause of death in these patients with a 16-fold to 19-fold increased risk of myocardial ischemia and infarction compared with control populations.[3] It has been suggested that homocysteine is a cause of cardiovascular disease in renal failure, and measures to reduce plasma homocysteine concentrations in this population have been advocated.[4]

Treatment with folic acid, a cosubstrate in the remethylation pathway of homocysteine, reduces plasma homocysteine in subjects with normal renal function by ≈30%.[5] Small uncontrolled studies have suggested that folic acid also lowers plasma homocysteine in patients with chronic renal failure.[4,6] However, the effect of folic acid on cardiovascular morbidity and mortality is unknown in either group. Endothelial dysfunction is the primary process in atherogenesis, and both in vitro and in vivo studies have suggested that this may be the mechanism of homocysteine induced vascular damage.[7–11] We hypothesized that in patients with renal failure, treatment with folic acid might reduce plasma homocysteine concentrations and thus reverse endothelial dysfunction. Evidence of such an effect would support the possibility that folic acid might be effective in reducing the burden of cardiovascular disease in patients with chronic renal failure.

In a double-blind, placebo-controlled, randomized trial, we have determined the effect of folic acid on endothelial function in patients with predialysis chronic renal failure. Endothelial function was assessed by measurement of (1) flow-mediated, endothelium-dependent dilation (EDD) of

Received December 22, 1999; revision received March 13, 2000; accepted March 16, 2000.

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TABLE 1. Definitions of Clinical Criteria for Presence of Atherosclerotic Vascular Disease and Risk Factors

Clinical Criteria	Definitions
Coronary artery disease	Any one of the following: documented history of myocardial infarction confirmed by Q waves on 12-lead ECG; typical anginal chest pain confirmed by evidence of inducible ischemia (exercise ECG or myocardial perfusion scan); angiographic evidence; coronary revascularization procedure
Cerebrovascular disease	Documented episode of sudden-onset neurological deficit
Peripheral vascular disease	Any of the following: documented history of intermittent claudication or abdominal aortic aneurysm; angiographic evidence; peripheral artery revascularization procedure; amputation for vascular disease
Renovascular disease	Angiographic evidence
Smoking history	Subjects who had smoked in the last year
Hypertension	Subjects taking antihypertensive therapy or with systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥90 mm Hg
Hypercholesterolemia	Subjects receiving lipid-lowering therapy or with fasting plasma total cholesterol >6.5 mmol/L
Family history	Presence of vascular disease in first-degree relative <65 y old

the brachial artery with the use of high-resolution ultrasound,[12] (2) combined serum nitrite and nitrate (NO_X) concentrations, stable end products of the nitric oxide radical,[13] and (3) plasma von Willebrand factor (vWF) concentration, a circulating marker of endothelial injury.[14]

Methods

Subjects

One hundred patients with chronic renal failure (serum creatinine >130 μmol/L) and a plasma homocysteine concentration >12 μmol/L were recruited from the predialysis clinic at the Queen Elizabeth Hospital, Birmingham, UK. The mean age of the group was 62 years (range 22 to 84), and there were 67 men. Exclusion criteria were atrial fibrillation, current or recent (within 6 months) use of folic acid supplements, B_{12} deficiency, or the presence of an arteriovenous fistula.

Study Design

The study was approved by the local research ethics committee, and written informed consent was obtained from all participants. Subjects were screened by a physician-administered questionnaire with the use of prospectively defined clinical criteria for the presence of atherosclerotic vascular disease and risk factors (Table 1). This was confirmed by a review of the hospital case notes. Measurements of waist-to-hip ratio and body mass index were noted, and after a 10-minute period of rest, blood pressure was recorded twice in the sitting position with the use of a standard sphygmomanometer. Brachial artery endothelial function was assessed, and fasting blood samples were obtained for measurement of serum urea and creatinine, lipid profile (total cholesterol, HDL cholesterol, and triglycerides), serum and red cell folate, serum vitamin B_{12}, plasma homocysteine, serum NO_X, and plasma vWF at baseline and after 12 weeks of treatment. Combined serum NO_X concentrations were measured by first generating serum nitrite from nitrate by enzymatic conversion with nitrate reductase. After deproteinization, total serum nitrite was then measured by means of the Griess reaction.[13] The intra-assay and interassay coefficients of variation are 6.6% and 9.2%, respectively. The glomerular filtration rate was calculated by means of the Cockcroft formula.[15] Plasma vWF was measured by enzyme-linked immunosorbent assay (Department of Rheumatology, University of Birmingham). Patients were randomized by computer to receive 5 mg folic acid or matching placebo once daily for 12 weeks. The dose of folic acid was chosen after a review of previous studies showed that doses of up to 5 mg lowered plasma homocysteine concentrations by ≈30% without adverse effects.[5] All investigators were blinded to the treatment allocation of the patients. Diet was not modified to maintain the usual dietary intake of folate.

Measurement of Plasma Homocysteine Concentration

Fasting blood samples were centrifuged within 20 minutes of collection and the plasma frozen at −70°C. Plasma total homocysteine was measured by ion-paired, reversed-phase high-performance liquid chromatography with electrochemical detection.[16] The reference value is 6 to 12 μmol/L, and the intra-assay and interassay coefficients of variation are 3.9% and 10.7%, respectively.

High-Resolution Ultrasonography of the Brachial Artery

After the discontinuation of vasoactive medication for 18 hours, EDD of the brachial artery, a nitric oxide–dependent process, was measured by ultrasound with standard techniques.[12,17] In each case, endothelium-independent dilation (EID), a reflection of vascular smooth muscle function, was also assessed by measuring the response to sublingual glyceryl trinitrate (GTN). Subjects were studied in the supine position at an ambient temperature of 20° to 23°C. A single investigator performed all imaging and analysis. A B-mode scan was obtained of the right brachial artery in longitudinal section between 5 and 12 cm proximal to the antecubital fossa with the use of a 7.5-MHz, phased-array transducer attached to a Sigma 44 HVD system (Kontron Instruments). Transducer positioning and depth and gain settings were adjusted to optimize the definition of anterior and posterior media-intima interfaces, which were used to demarcate the brachial artery diameter. This diameter was calculated as the average of measurements made during 4 cardiac cycles at end-diastole. All measurements were recorded on super-VHS videotape for subsequent off-line analysis. Each study comprised a series of artery diameter measurements as follows: (1) at rest after a 10-minute period of acclimatization; (2) EDD 60 to 90 seconds after the sudden deflation of a pneumatic cuff placed on the ipsilateral forearm that had been inflated to suprasystolic pressure for 5 minutes; (3) second resting diameter after a 10-minute recovery period; and (4) EID 4 minutes after sublingual administration of 800 μg GTN spray. Endothelium-dependent and endothelium-independent dilation were expressed as the percent change from the mean resting artery diameter, calculated from the average of the first and second resting recordings. The repeatability (intraobserver variability) of this technique was calculated from measurements obtained from 17 subjects by the investigator. The mean (SD) relative difference in the measurements made on 2 separate occasions was 2.4% (2.1), 3.7% (3.9), and 3.2% (2.5) for the average baseline diameter, EDD, and EID, respectively.

Statistical Power and Analysis

A sample size of 90 patients in this parallel group design had an 80% power (at $\alpha=0.05$) to detect a difference in EDD of 2%.[18] One hundred patients were recruited to ensure an adequately powered study, with a dropout rate up to 10%. Data were analyzed with the

TABLE 2. Baseline Characteristics of Folic Acid and Placebo Groups

	Folic Acid (n=50)	Placebo (n=50)
Age, y	61 (57–64)	62 (59–66)
Sex, M:F	37:13	36:14
Smoker, never/ex/current, n	16/29/5	16/28/6
Hypertension, n	44	46
Systolic BP, mm Hg	146 (139–153)	155 (148–161)
Diastolic BP, mm Hg	86 (81–90)	88 (85–92)
Hypercholesterolemia, n	20	22
Total cholesterol, mmol/L	5.3 (5.0–5.7)	5.6 (5.3–6.0)
Triglycerides, mmol/L	2.01 (1.73–2.32)	1.94 (1.62–2.32)
HDL cholesterol, mmol/L	1.14 (1.04–1.24)	1.17 (1.05–1.30)
LDL cholesterol, mmol/L	3.0 (2.8–3.3)	3.4 (3.0–3.7)
Family history, n	19	20
Vascular history, n	25	24
Diabetes mellitus, n	6	8
BMI, kg/m^2	28.2 (26.6–29.8)	27.5 (26.1–28.8)
WHR	0.94 (0.92–0.96)	0.92 (0.90–0.94)
Urea, mmol/L	15.5 (13.9–17.4)	16.0 (14.1–18.2)
Creatinine, μmol/L	245 (221–271)	257 (228–290)
GFR, mL/min	30.1 (26.4–34.4)	27.5 (23.7–31.7)
Serum folate, μg/L	6.9 (5.9–8.0)	7.7 (6.5–9.2)
Red cell folate, μg/L	207 (184–235)	199 (172–229)
Vitamin B_{12}, ng/L	434 (381–496)	457 (397–526)
Plasma homocysteine, μmol/L	17.7 (16.3–19.2)	18.5 (16.8–20.3)
Serum nitrate+nitrite, μmol/L	50.7 (44.2–58.3)	44.0 (39.0–49.6)
Plasma vWF, IU/dL	264 (247–281)	250 (232–267)
Resting diameter, cm	0.50 (0.48–0.52)	0.50 (0.47–0.53)
EDD, %	3.7 (2.8–4.6)	2.6 (1.7–3.5)
EID, %	11.2 (9.7–12.7)	10.1 (8.8–11.4)

BMI indicates body mass index; BP, blood pressure; GFR, glomerular filtration rate; and WHR, waist-to-hip ratio.

Continuous variables are expressed as mean (95% CI). There were no significant differences between the 2 groups as assessed by χ^2 and unpaired *t* tests as appropriate. LDL cholesterol was calculated from the Friedlwald equation.

use of SPSS for Windows 9.0. Means and 95% confidence intervals were used to describe continuous variables. Variables that were not normally distributed were log-transformed. The distributions of discrete and continuous variables between groups were compared by means of χ^2 and unpaired *t* tests. Linear regression was used to assess the association between potential predictor variables and measures of endothelial function. The test results are presented as 2-tailed values, and statistical significance was inferred at $P<0.05$.

Results

There were no significant differences in the baseline characteristics of the folic acid and placebo groups (Table 2). None of the patients had preexisting deficiencies in folate or vitamin B_{12}. Three patients from the folic acid group and 6 patients from the placebo group were withdrawn from the trial. Reasons for this were commencement of hemodialysis (3 patients), failure to attend the posttreatment visit (3 patients), sudden death (1 patient), and discontinuation of treatment after a new skin rash (1 patient in each group). All other patients tolerated folic acid without side effects. The only significant difference in baseline characteristics between patients withdrawn from the study and those who completed the course of treatment was a higher mean serum creatinine [400 (95% CI 279 to 575) versus 240 (223 to 258) μmol/L; $P<0.05$].

TABLE 3. Posttreatment Measures of Endothelial Function

	Folic Acid	Placebo
Resting diameter, cm	0.49 (0.47–0.51)	0.50 (0.48–0.53)
EDD, %	4.3 (3.5–5.2)	3.9 (2.9–5.0)
EID, %	12.2 (10.6–13.8)	10.4 (8.7–12.1)
Serum nitrite+nitrate, μmol/L	47.8 (42.6–53.6)	44.8 (38.4–52.1)
Plasma vWF, IU/dL	281 (257–305)	276 (250–302)

Continuous variables are expressed as mean (95% CI). Significance was assessed by unpaired *t* tests.

After 12 weeks of treatment, serum folate concentration was significantly greater in the folic acid group than the placebo group [39.0 (95% CI 29.8 to 51.0) versus 7.7 (6.6 to 8.9) μg/L; $P<0.001$], as was red cell folate concentration [739 (613 to 891) versus 220 (184 to 262) μg/L; $P<0.001$]. The plasma homocysteine concentration in the folic acid group was significantly lower than in the placebo group [15.1 (14.1 to 16.2) versus 20.1 (18.2 to 22.2) μmol/L; $P<0.001$]. This represented a 24.9% reduction in plasma homocysteine in the folic acid group compared with the placebo group. At the end of the treatment period, plasma homocysteine concentration was <12 μmol/L in 20% of the folic acid group and 5% of the placebo group. There was no significant increase in either serum creatinine or glomerular filtration rate in either group during the duration of the study.

Treatment with folic acid was not associated with a significant improvement in EDD, EID, or serum NO_X concentration compared with placebo (Table 3). In addition, there was no significant difference in plasma vWF between the 2 groups (Table 3). These findings were not altered by the exclusion of current smokers, diabetics, or patients with clinical evidence of atherosclerotic vascular disease. The absolute reduction in plasma homocysteine correlated strongly with the absolute increase in both serum and red cell folate concentrations [$r=-0.343$; $P<0.01$ and $r=-0.449$; $P<0.01$, respectively]. There were no correlations between changes in EDD, EID, serum NO_X concentration, or plasma vWF and changes in either folate status or homocysteine levels.

Discussion

This study demonstrates that treatment with high-dose folic acid results in a significant reduction in plasma homocysteine but no improvement in endothelial function in patients with chronic renal failure. Although no directly comparable studies have been conducted, a small open-label trial of folic acid at doses up to 15 mg daily for 1 year in hemodialysis patients also showed no improvement in EDD or plasma vWF concentration.[19] However, in healthy

volunteers, impaired EDD during transient acute hyperhomocysteinemia was prevented by the coadministration of folic acid.[11] In addition, a reduction in plasma vWF concentrations after treatment with folic acid and pyridoxine for 1 year has been reported in a small nonblinded study of patients with peripheral vascular disease.[20] Further study of the effect of folic acid on endothelial function in nonuremic patients with hyperhomocysteinemia is required.

There are a number of possible explanations for our findings. The chronic exposure to elevated homocysteine concentrations or other as yet undefined atherogenic influences in uremia may result in early and irreversible endothelial damage. We have previously demonstrated that endothelial dysfunction is present in patients with biochemically mild renal insufficiency,[21] thus therapy may need to be directed at patients at a much earlier "subclinical" stage of progressive renal failure.

It is also possible that greater reductions in plasma homocysteine are required to correct endothelial dysfunction in chronic renal failure. Despite increases in serum folate to twice the upper limit of the reference range, normal plasma homocysteine concentrations (<12 μmol/L) were achieved in only 20% of treated patients so that the vascular endothelium remained exposed to supraphysiological levels of homocysteine. Although plasma homocysteine concentrations in nonuremic subjects can often be normalized by low doses of folic acid,[5] such reductions have not been consistently achieved with up to 15 mg of folic acid per day in patients with chronic renal failure.[4,6,19,22] The metabolism of homocysteine is also dependent on other micronutrients, including the B-group vitamins. However, correction of hyperhomocysteinemia in uremia has not been demonstrated with vitamins B_6, B_{12},[6] serine,[23] or betaine.[19] In a placebo-controlled trial, a combination of folic acid (15 mg/d) and vitamins B_6 and B_{12} normalized plasma homocysteine in only one third of dialysis patients.[24] Further work is needed to determine the optimal combination and doses of micronutrients required to lower homocysteine in patients with chronic renal failure. Furthermore, studies in vitro[25] and in vivo[26,27] have suggested that increased oxidant stress is the principal mechanism of homocysteine induced endothelial toxicity. Thus, strategies to improve endothelial function in hyperhomocysteinemia may require the addition of antioxidants.

The duration of treatment required to reverse endothelial dysfunction is unclear. However, it is known that treatment with folic acid for 1 year provided no additional reduction in plasma homocysteine or improvement in endothelial function compared with a shorter course.[19] This finding and the demonstration of improved endothelial function 1 month after commencing cholesterol-lowering agents in patients with hypercholesterolemia[28] suggest an adequate duration of treatment in this study. Finally, the multiple metabolic abnormalities associated with renal failure may interact to induce endothelial injury; thus, targeting hyperhomocysteinemia alone may be insufficient to reverse this process.

In conclusion, this study demonstrates that high-dose folic acid lowers but fails to normalize plasma homocysteine concentration in patients with predialysis chronic renal failure. Partial correction of hyperhomocysteinemia was not accompanied by an improvement in endothelial function. These results do not support the routine use of folic acid in the prevention and treatment of vascular disease in this high-risk group.

Acknowledgments

This work was funded by a project grant from the British Heart Foundation, which supported Dr Thambyrajah and Dr Townend. We would like to thank the patients, doctors, and staff at the Department of Nephrology, Queen Elizabeth Hospital, Birmingham, UK, for all their help in enabling us to complete this study. We would also like to thank the Department of Rheumatology, University of Birmingham, for measuring plasma von Willebrand factor concentration and the Clinical Investigation Unit, University of Birmingham, for measuring combined nitrite concentrations.

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Circulation. 2000;102:871-875
doi: 10.1161/01.CIR.102.8.871
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539