Effects of Oral Folic Acid Supplementation on Endothelial Function in Familial Hypercholesterolemia
A Randomized Placebo-Controlled Trial

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Background—Folates have been suggested to be of benefit in reducing cardiovascular risk. The present study was designed to examine whether oral folic acid supplementation could improve endothelial function as an intermediate end point for cardiovascular risk in patients with increased risk of atherosclerosis due to familial hypercholesterolemia (FH).

Methods and Results—In a prospective, randomized, double-blind, placebo-controlled study with crossover design, we evaluated the effects of 4 weeks of treatment with oral folic acid (5 mg PO) on endothelial function in FH. In 20 FH patients, forearm vascular function was assessed at baseline, after 4 weeks of folic acid treatment, and after 4 weeks of placebo treatment by venous occlusion plethysmography, with serotonin and sodium nitroprusside used as endothelium-dependent and -independent vasodilators. In addition, we examined the vasoconstrictor response to the NO synthase inhibitor \( \text{N}^G \text{-monomethyl-L-arginine} \) to assess basal NO activity. In FH patients, folic acid supplementation restored the impaired endothelium-dependent vasodilation, whereas it did not significantly influence endothelium-independent vasodilation or basal forearm vasomotion. There was a trend toward improvement in basal NO activity.

Conclusions—These data demonstrate that oral supplementation of folic acid can improve endothelial function in patients with increased risk of atherosclerotic disease due to hypercholesterolemia, without changes in plasma lipids. (Circulation. 1999;100:335-338.)

Key Words: endothelium ■ nitric oxide ■ folate ■ hypercholesterolemia

Recently, low serum folate levels were associated with increased risk of cerebrovascular and coronary artery disease.\(^1,2\) In addition, a common mutation of 5,10-methylenetetrahydrofolate reductase (MTHFR), which causes reduced formation of 5-methyltetrahydrofolate (5-MTHF, the active form of folate), has been reported to be a risk factor for vascular disease, very likely also depending on folate status.\(^3\)

Endothelial dysfunction is an early sign of atherosclerotic disease. Impaired endothelium-dependent, NO-mediated vasodilation could be demonstrated in patients with cardiovascular risk factors, such as hypercholesterolemia and hyperhomocysteinemia. Folic acid therapy has been shown to improve endothelial function in hyperhomocysteinemia. We recently demonstrated that acute intra-arterial administration of the active form of folate could also restore the impairment in endothelial function in patients with increased risk of atherosclerotic disease but normal serum folate and homocysteine levels.\(^4\)

The present study was designed to determine whether this concept can be extrapolated to clinical practice and thus whether oral folic acid supplementation can improve endothelial function in patients with increased cardiovascular risk due to familial hypercholesterolemia (FH) without hyperhomocysteinemia. Such a beneficial effect of folate therapy would be of great clinical relevance, because folic acid is inexpensive and nontoxic and can be safely prescribed. Therefore, in a prospective, randomized, double-blind, crossover study, we investigated the effects of 4 weeks of treatment with 5 mg oral folic acid supplementation compared with placebo on endothelial function as an intermediate end point for cardiovascular risk.

Methods

Subjects
Twenty patients with FH participated in our study. Baseline measurements were performed after \( \geq 2 \) weeks of withdrawal of lipid-lowering medication. Baseline endothelial function in FH patients was compared with measurements of endothelial function in 20 healthy control subjects matched for age, sex, and smoking habit. None of the participants in the study had clinical signs of cardiovas-
cular disease. All subjects abstained from alcohol, tobacco, and caffeine-containing drinks for ≥12 hours before measurements were made.

**Study Design**

The study protocol was approved by the local research ethics committee of the University Hospital Utrecht. The investigations conformed with the principles outlined in the Declaration of Helsinki. After baseline assessment of endothelial function, patients were randomized in a double-blind manner to receive either placebo for a period of 4 weeks followed by folic acid treatment (5 mg PO) for 4 weeks, or vice versa. After each treatment period, endothelial function was assessed. During the study (10 weeks in total), patients were not using any lipid-lowering medication.

**Study Protocol**

Forearm blood flow (FBF) was measured simultaneously in both arms by venous occlusion plethysmography. Serotonin (Sigma) and sodium nitroprusside (Merck) were infused, in random order, into the nondominant brachial artery to assess endothelium-dependent and -independent vasodilation as described previously. These serotonin dosages have previously been shown to cause specific NO-mediated vasodilation. In addition, we subsequently assessed the vasoconstrictor response to increasing doses of the NO synthase inhibitor N^G- monomethyl-L-arginine (L-NMMA; Institut für Pharmazie, Universität Leipzig; 8 minutes per dose) to estimate basal NO activity (see Figure).

Tetrahydrobiopterin (BH_{4}) levels were determined by reverse-phase high-performance liquid chromatography. Other biochemical parameters were measured as described previously.

**Analysis**

The hypothesis of the study was that treatment with folic acid for 4 weeks would result in improved endothelial function compared with placebo. A sample size of 16 patients was necessary to achieve 80% treatment effect with a 2-sided 5% significance. The ratio of flows in the infused and noninfused arms (M/C ratio) was calculated for each time point and expressed as percentage change from baseline. Results are expressed as mean±SEM. Differences were examined by repeated-measures ANOVA (Jandel Scientific Inc). If variance ratios reached statistical significance, differences between the means were analyzed with the Student-Newman-Keuls test with a significance level of P<0.05. Baseline characteristics of patients and control subjects were compared by a t test or, when normality test failed, Mann-Whitney rank-sum test and χ² test.

**Results**

Patient characteristics and laboratory data are shown in the Table. Lipid profiles were not different between the placebo- and the folic acid–treated groups. Oral folic acid supplementation increased both serum and red cell folate (P<0.05 versus placebo for both) and decreased plasma homocysteine levels (P<0.05).

**Effects of Oral Folic Acid Supplementation on Baseline FBF**

Treatment with folic acid did not alter baseline FBF in either the infused or the noninfused arm. Accordingly, M/C ratios were unaltered by folic acid treatment. No effects on mean arterial pressure or heart rate were observed (Table).

**Effects of Oral Folic Acid Supplementation on Endothelium-Dependent Vasodilation**

Serotonin-induced vasodilation was impaired in hypercholesterolemic patients at baseline; M/C ratio increased from 1.04±0.04 to 1.63±0.1 versus 1.24±0.1 to 2.37±0.18 in control subjects (P<0.05). Four weeks of treatment with placebo did not alter endothelium-dependent vasodilation (1.09±0.07 to 1.58±0.14 increase in M/C ratio, NS versus baseline), whereas 4 weeks of oral folic acid supplementation (5 mg PO) enhanced serotonin-induced vasodilation (M/C ratio from 1.02±1.89 to 1.89±0.16, P<0.05 versus placebo). There was no difference in serotonin-induced vasodilation between patients on folic acid treatment and normocholesterolemic control subjects (Figure).

**Effects of Oral Folic Acid Supplementation on Endothelium-Independent Vasodilation**

Sodium nitroprusside infusion caused increases in FBF, which were not different between FH patients at baseline,
Effect of Oral Folic Acid Supplementation on the Vasoconstrictor Response to L-NMMA

In the placebo group, infusion of incremental doses of the NO synthase inhibitor L-NMMA caused a 36±5% decrease in M/C ratio (1.12±0.07 to 0.70±0.06). After folic acid treatment, the vasoconstrictor response to L-NMMA was slightly, but not significantly, increased, causing a 45±4% decrease in M/C ratio (1.24±0.10 to 0.65±0.04) (Figure).

Discussion

This placebo-controlled, randomized, double-blind study shows that 4 weeks of oral folic acid therapy (5 mg PO) restores impaired endothelial function in patients with increased cardiovascular risk due to familial hypercholesterolemia. This beneficial effect occurs without changes in plasma lipid levels. We also found a nonsignificant trend toward improvement in basal NO activity.

Folic acid supplementation has previously been shown to have a beneficial effect on endothelial function in patients with hyperhomocysteinemia, measured as flow-mediated vasodilation or estimated as plasma markers of endothelial dysfunction. Such a homocysteine-lowering effect may also have contributed to the observed improvement of endothelial function in our population. However, additional mechanisms are likely to be involved, considering the relatively modest effect on homocysteine levels that were already in the normal range and considering the fact that in our previous experiments, acute administration of the active form of folic acid also improved endothelial function without any effects on homocysteine levels.

Folates have been suggested to be involved in endogenous regeneration of BH4, an essential cofactor for NO synthase. We recently demonstrated that administration of BH4 could restore endothelial function in hypercholesterolemia. However, our data do not show any increase in plasma biotin levels after folate supplementation. Although an increase in BH4 tissue levels cannot be entirely excluded, the present findings make this mechanism less likely.

Alternatively, an antioxidant effect of folates may explain their beneficial effect on endothelial function. It has now been recognized that enhanced oxidative degradation of NO is an important determinant of endothelial dysfunction in hypercholesterolemia, as well as in other risk factors associated with endothelial dysfunction. Recent in vitro data suggest a direct antioxidant effect of folates, but indirect antioxidant effects are also possible, such as improvement of the cellular antioxidant defense system. However, whether reduction of vascular oxidative stress is an important mechanism in vivo cannot easily be determined, because reliable methods to assess oxidant stress are still lacking.

Our data suggest that oral folic acid therapy may provide a safe and inexpensive tool to reduce cardiovascular risk, not only in patients with elevated homocysteine levels but also in hypercholesterolemia. The present observation may have important clinical implications, particularly in hypercholesterolemic patients who do not respond sufficiently to lipid-lowering medication; in hypercholesterolemic patients in whom lipid-lowering medication is not recommended, such as children or women of childbearing age; or as adjuvant therapy.
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References
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