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Short-Term Oral Folic Acid Supplementation Enhances Endothelial Function in Patients With Type 2 Diabetes

Arduino A. Mangoni, Roy A. Sherwood, Belinda Asonganyi, Cameron G. Swift, Stephen Thomas, and Stephen H.D. Jackson

Background: Endothelial dysfunction and arterial stiffening are commonly observed in type 2 diabetes. These abnormalities might be secondary to increased plasma concentrations of homocysteine. We sought to determine whether oral folic acid supplementation, by lowering homocysteine levels, enhanced endothelial function and reduced arterial stiffness in type 2 diabetes.

Methods: Twenty-six type 2 diabetic patients (age 56.5 ± 0.9 years, diabetes duration 5.5 ± 0.6 years, means ± SEM) with no history of cardiovascular disease received 5 mg/d of oral folic acid or placebo for 4 weeks in a double-blind, randomized controlled, parallel group trial. The following parameters were measured before and after treatment: 1) endothelial function (forearm arterial blood flow during local intra-arterial administration of endothelium-dependent [acetylcholine 1.5, 4.5, and 15 μg/min] and endothelium-independent [sodium nitroprusside 1, 2, and 4 μg/min] vasodilators); and 2) carotid–radial and carotid–femoral pulse wave velocity.

Results: Folic acid reduced plasma homocysteine concentrations and enhanced endothelium-dependent vasodilatation during each acetylcholine infusion rate (mean and 95% confidence interval post versus pretreatment differences in forearm arterial blood flow ratio between the infused and control arm +0.19 (0.03–0.35), $P < .01$; +0.39 (0.02–0.81), $P < .05$; and +0.40 (0.09–0.89), $P < .05$, respectively). Endothelium-independent vasodilatation and pulse wave velocity were not affected. No significant changes in forearm arterial blood flow and pulse wave velocity were observed in the placebo group. Multiple regression analysis showed that changes in folic acid, but not homocysteine, concentrations independently described changes in maximal endothelium-dependent vasodilatation.

Conclusions: Short-term oral folic acid supplementation significantly enhances endothelial function in type 2 diabetic patients, independent of homocysteine lowering. Am J Hypertens 2005;18:220–226 © 2005 American Journal of Hypertension, Ltd.

Key Words: Folic acid, homocysteine, endothelium, type 2 diabetes.

Type 2 diabetes mellitus is characterized by early development of atherosclerosis. Although tight control of blood pressure (BP), blood glucose, and serum lipids reduce cardiovascular risk, cardiovascular morbidity and mortality rates remain unacceptably high and the risk factor management is notoriously difficult in these patients.[1–3] Therefore, the identification of alternative strategies to reduce cardiovascular risk in patients with type 2 diabetes is urgently needed.

Endothelial dysfunction and arterial stiffening are common findings in type 2 diabetes.[4,5] This might explain, at least partly, the increased cardiovascular morbidity and mortality observed in this condition.[6,7] Increased plasma concentrations of homocysteine, a highly reactive sulfur-containing amino acid, are associated with impaired endothelial function and arterial stiffening, the same abnormalities observed in type 2 diabetes.[8,9] Moreover, plasma homocysteine concentrations represent a strong and independent predictor of cardiovascular morbidity and mortality in this population.[10]

Plasma concentrations of the B-vitamin folic acid are inversely correlated with homocysteine plasma concentra-

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From the Department of Clinical Pharmacology, Centre for Neuroscience, Flinders University, School of Medicine (AAM), Adelaide, Australia; and Departments of Health Care of the Elderly (AAM, CGS, SHDJ), Clinical Biochemistry (RAS, BA), Diabetic Medicine (ST), Guy's, King's, and St Thomas' School of Medicine, King's College, London, United Kingdom.

Address correspondence and reprint requests to Dr. Arduino A. Mangoni, Department of Clinical Pharmacology, Flinders Medical Centre, Bedford Park, SA 5042, Australia; e-mail: arduino.mangoni@flinders.edu.au

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tions.[11] Oral folic acid supplementation safely and effectively lowers homocysteine concentrations within 4 to 6 weeks.[12,13] We have previously observed reductions between 17% and 24% in homocysteine concentrations with such a regimen.[14] Therefore, folic acid supplementation could potentially mitigate the vascular abnormalities observed in type 2 diabetes. The aim of our study was to investigate the effects of oral folic acid supplementation for 4 weeks on endothelial function and arterial stiffness in patients with type 2 diabetes and no previous cardiovascular events.

Methods

Subjects

Twenty-six patients with type 2 diabetes (aged 56.5 ± 0.9 years, range 46 to 65 years; diabetes duration 5.5 ± 0.6 years, means ± SEM) were recruited from diabetic and general medical outpatient clinics and local advertising. The subjects had no history of angina, myocardial infarction, stroke, or peripheral occlusive disease. Hypertension was present in 16 patients. One patient was on 4 antihypertensive drugs, 3 patients were on 3, 4 patients were on 2, and 8 patients were on 1 drug. Antihypertensive treatment included diuretics in 6 patients, angiotensin converting enzyme inhibitors in 7, angiotensin II receptor antagonists in 3, β-blockers in 7, calcium channel blockers in 4, and α-blockers in 2 patients. Antidiabetic treatment included oral hypoglycemic agents and insulin in 4 patients, oral hypoglycemic agents alone in 19, insulin alone in 2, and diet alone in 1 patient. Microalbuminuria, defined as urinary albumin:creatinine ratio ≥2.5 mg/mmol (men) or ≥3.5 mg/mmol (women), was present in 8 subjects. The study was approved by the local Research Ethics Committee. Each subject gave written informed consent before starting the study.

Protocol

Investigations were performed in a temperature-controlled laboratory (25° to 27°C). The subjects were asked to abstain from cigarette smoking and alcohol consumption from the evening before the study. Each subject underwent three visits. During visit 1, a physical examination and an electrocardiogram were performed, BP (mean of three consecutive readings after the subject was resting for 5 min) and heart rate (HR) were measured, and blood and urine samples were taken (cholesterol, folic acid, vitamin B_{12} concentrations, and urinary albumin:creatinine ratio). During visit 2, another blood sample was taken (full blood count, biochemistry, fasting homocysteine, folic acid, and vitamin B_{12}) and the following parameters were measured: forearm arterial blood flow (FABF), pulse wave velocity (PWV), and BP. Then, the subjects were randomly assigned to 4-week treatment with either folic acid (5 mg/d) or identically appearing placebo. The allocation sequence was generated by software located in the Pharmacy Department. Both the investigators and the subjects were blinded to the treatment. The subjects returned after 4 weeks for visit 3, for the reassessment of the parameters measured during visit 2. Both FABF and PWV studies were performed by the same investigator (AAM). Vasodilators were stopped 5 days before each study day. This washout period was considered adequate as the elimination half-life of vasodilators ranged between 11 and 22 hours.

FABF

Endothelial function was assessed by the perfused forearm technique. The brachial artery was cannulated using a 27-gauge cannula connected by an epidural catheter to a constant infusion pump. Forearm arterial blood flow was measured simultaneously in both arms (infused and control forearm) by strain-gauge venous occlusion plethysmography (D.E. Hokanson Inc., Bellevue, WA). Baseline measurements were obtained after each subject rested supine for 20 min. Then, endothelial function was assessed by 8-min intra-arterial infusions of incremental doses of an endothelium-dependent vasodilator (acetylcholine 1.5, 4.5, and 15 μg/min; Clinalfa, Laeufelfingen, Switzerland). After a second baseline was obtained, an endothelium-independent vasodilator was administered (sodium nitroprusside 1, 2, and 4 μg/min; David Bull Laboratories, Warwick, UK). The order of acetylcholine and sodium nitroprusside infusion was randomized. The doses of vasodilators used did not have any systemic effect on BP and HR. The FABF measurements were taken during the final 2 min of each infusion rate. Circulation to the hands was excluded 1 min before FABF measurement by inflating a pediatric cuff around the wrist at suprasystolic BP.

PWV

Carotid–radial and carotid–femoral PWV were measured noninvasively by a Complior (Colson, Createch Industrie, Garges les Gonesse, France). This device consists of two acoustic sensors, which detect the pulse wave signals and deliver them to a computerized data acquisition board. The latter samples the data at a frequency of 800 Hz and calculates the transit time between the common carotid and radial or femoral arteries. The measurement of the distance between these points allows the calculation of PWV.[15] Twenty consecutive measurements from the left limbs were performed during each visit and averaged. The coefficient of variation of two previous sets of measurements performed in 7 healthy subjects by the same operator 24 h apart averaged 2.3%.

Homocysteine, Folic Acid, and Vitamin B_{12}

Fasting venous blood samples were collected into tubes containing disodium EDTA and tubes without anticoagulation. The samples were centrifuged at 1800 *g* within 30 min, the plasma/serum separated and stored at −20°C.