Treatment of Mild Hyperhomocysteinemia in Vascular Disease Patients

Diana G. Franken, Godfried H.J. Boers, Henk J. Blom, Frans J.M. TrijbeIs, Peter W.C. Kloppenborg

Abstract Mild hyperhomocysteinemia is recognized as a risk factor for premature arteriosclerotic disease. A few vitamins and other substances have been reported to reduce blood homocysteine levels, but normalization of elevated blood homocysteine concentrations with any of these substances has not been reported. Therefore, we screened 421 patients suffering from premature peripheral or cerebral occlusive arterial disease by oral methionine loading tests for the presence of mild hyperhomocysteinemia. Thirty-three percent of patients with peripheral and 20% of patients with cerebral occlusive arterial disease were identified with mild hyperhomocysteinemia (14% of the men, 34% of the premenopausal women, and 26% of the postmenopausal women). Mildly hyperhomocysteinemic patients were administered vitamin B6, 250 mg daily. After 6 weeks methionine loading tests were again assessed to evaluate the effect of treatment. Patients with nonnormalized homocysteine concentrations were further treated with vitamin B6, 250 mg daily and/or folic acid 5 mg daily and/or betaine 6 g daily, solely or in any combination. Vitamin B6 treatment normalized the afterload homocysteine concentration in 56% of the treated patients (71% of the men, 45% of the premenopausal women, and 88% of the postmenopausal women). Further treatment resulted in a normalization of homocysteine levels in 95% of the remaining cases. Thus, mild hyperhomocysteinemia, which is frequently encountered in patients with premature arteriosclerotic disease, can be reduced to normal in virtually all cases by safe and simple treatment with vitamin B6, folic acid, and betaine, each of which is involved in methionine metabolism. (Arterioscler Thromb. 1994;14:465-470.)

Key Words • hyperhomocysteinemia • vitamin B6 • folic acid • betaine • vascular disease

Classic homocystinuria, due to homozygosity for cystathionine synthase (CS) deficiency, is characterized by severe accumulation of homocysteine in the blood and tissues. The incidence of this hereditary dysfunction varies geographically and is estimated as 1:200 000 worldwide.1 Homocystinuria is generally considered to cause premature arteriosclerosis and thromboembolism. It is treated with high-dose administration of vitamin B6, the active form of which, pyridoxal phosphate, functions as a cofactor in the conversion of homocysteine to cystathionine (Fig 1). The marked homocysteine-lowering effect of vitamin B6 is attributable to its stimulation of the residual activity of the cystathionine synthase enzyme.1-4 Additionally, folic acid and betaine, both involved in the remethylation of homocysteine into methionine, can lower or even normalize elevated homocysteine levels in patients who respond poorly or not at all to vitamin B6 treatment.1,3 The incidence of vascular accidents is significantly reduced after initiating homocysteine-lowering treatment, revealing the clinically beneficial effect of such intervention in homocystinuric patients.4

Mild hyperhomocysteinemia, with homocysteine concentrations equivalent to those found in individuals heterozygous for CS deficiency, is characterized by mildly elevated blood homocysteine concentrations in the fasting state or after standardized methionine loading. However, intermediate CS deficiency is not the only possible genetic determinant of mild hyperhomocysteinemia. The occurrence of a mutant variant of methylenetetrahydrofolate reductase, characterized by 50% of normal activity and thermolability of the enzyme, has also been described in vascular disease patients with mild hyperhomocysteinemia.5 Overall, in 9% to 42% of the patients under 50 years of age suffering from peripheral or cerebral occlusive arterial disease, myocardial infarction, or thromboembolism, mild hyperhomocysteinemia has been observed and has come to be recognized as an independent risk factor for premature arteriosclerotic disease.6-24 Routine screening for mild hyperhomocysteinemia among patients with signs of premature arteriosclerosis or thromboembolism at a young age is recommended if elevated homocysteine levels can be reduced by a safe and simple regimen that will produce a beneficial clinical effect. The homocysteine-lowering effect of vitamin B6, folic acid, or betaine has been observed in small groups of patients suffering from arteriosclerotic disease.22,23 However, normalization of elevated homocysteine concentrations after methionine loading in large groups of hyperhomocysteinemic arteriosclerotic patients treated with high-dose administration of one or more of these compounds has not been reported. The present study not only included a large number of hyperhomocysteinemic patients, all suffering from premature peripheral or cerebral occlusive arterial disease, but, more importantly, also considered the homocysteine-normalizing effect of high-dose administration of vitamin B6, folic acid, and betaine.
At the time of these studies the determination of the free homocysteine concentrations was routinely performed, which left the fasting levels unmanageably low. Therefore, only peak levels of homocysteine after methionine loading could be included. The introduction of the technique to measure total homocysteine, ie, free plus protein bound, in our laboratory in 1991 (after completion of this study) allowed a more sensitive determination of homocysteine blood levels in the fasting state. However, future studies will have to prove the adequacy of sensitivity and specificity of fasting total homocysteine levels for the establishment of a mildly hyperhomocysteinemic state in the individual vascular disease patient.

Methods

Study Population

During the period January 1980 through December 1990, 421 patients under 55 years of age with documented premature peripheral or cerebral occlusive arterial disease were screened for mild hyperhomocysteinemia by means of an oral methionine loading test. The diagnosis of cerebral infarction was documented by cerebral computerized tomography scanning in all these patients. Patients with known risk factors such as diabetes (fasting plasma levels more than 7.3 mmol/L), hyperlipoproteinemia (fasting serum levels of cholesterol more than 6.3 mmol/L and triglycerides more than 2.0 mmol/L), and hypertension (systolic and diastolic blood pressure more than 150 mm Hg and 95 mm Hg, respectively) of nonrenovascular origin were excluded from the study. In all patients the blood levels of vitamin B6 (normal range, 28 to 107 nmol/L), B12 (160 to 750 pmol/L), folate acid (5.5 to 40.0 nmol/L), creatinine, and the liver enzymes aspartate aminotransaminase and alanine aminotransaminase were within normal ranges. Of the included patients, 131 (58 men and 73 women) had peripheral occlusive arterial disease that had led to intermittent claudication or renovascular hypertension, and 290 (145 men and 145 women) had cerebral occlusive arterial disease with a variety of persistent or transient neurological signs (eg, hemiplegia and aphasia). All patients originated from different kindreds. The effect of homocysteine-lowering treatment could be evaluated retrospectively in 82 hyperhomocysteinemic patients. The 21 men, 53 premenopausal women, and 8 postmenopausal women were, respectively, 42±8 (mean±1 SD) years (range, 16 to 55), 39±6 years (range, 24 to 50), and 50±3 years (range, 46 to 55).

In 63 control subjects a methionine loading test was performed to establish the normal range of postload homocysteine levels. All control subjects were without vascular complications or any medication, and in all the vitamin B6, B12, and folic acid concentrations were within the normal ranges. The control men (n=20), premenopausal women (n=33), and postmenopausal women (n=10) were, respectively, 42±11 (mean±1 SD) years (range, 22 to 55), 32±10 years (range, 16 to 54), and 53±3 years (range, 45 to 55). Postmenopausal status was established by questionnaire (no menses for more than 1 year) and clinically ascertained by estrogen level.

Methionine Loading Test and Mild Hyperhomocysteinemia

The methionine loading test was performed after an overnight fast. L-Methionine at a dose of 0.1 g/kg body weight was administered orally in orange juice. During the next 8 hours the patient received a meal containing 14 mg of methionine. Four, 6, and 8 hours after methionine loading, blood samples were drawn. The blood samples were immediately centrifuged, and the serum was deproteinized and stored at −20°C until analysis. Free homocysteine concentrations were determined in all blood samples as homocysteine and homocysteine-cystine mixed disulfide concentrations by ion-exchange chromatography (Biotronik I C 2000 amino acid analyzer).6,26,27 Patients were considered to be hyperhomocysteinemic if their homocysteine peak level after methionine loading exceeded the mean peak level plus 2 SD in the group of control subjects. Because of observed differences in mean homocysteine peak levels after methionine loading between control men, premenopausal women, and postmenopausal women, the studied patients were categorized accordingly.6,26,27 The peak in postload homocysteine levels occurred in 87% of all performed tests at 6 hours, in 6% at 4 hours, and in 7% at 8 hours after loading.

Protocol of Homocysteine-Lowering Treatment

Patients identified as hyperhomocysteinemic received vitamin B6 (pyridoxine hydrochloride) 250 mg PO daily. Six weeks...
later, with treatment continuing, a second methionine loading test was performed to assess the homocysteine-lowering effect. Because the aim of treatment was to normalize the elevated homocysteine level, we defined the reduction $R$ by the formula

$$R = \left(1 - \frac{L_2 - L_c}{L_1 - L_c}\right) \times 100\%$$

where $L_1$ is homocysteine peak level after methionine loading before treatment, $L_2$ is homocysteine peak level after methionine loading while on treatment, and $L_c$ is mean homocysteine peak level after methionine loading plus 2 SD in control subjects.

We defined a reduction of $L_2$ to $L_c$ (or a lower value) as "normalization," a reduction of less than 60% as a "poor or nonresponse," and a reduction of between 60% and 99% as an "intermediate response." This classification of the patient's response determined the mode of further treatment. In hyperhomocysteinemic patients in whom vitamin B₆ therapy failed to achieve normalization of the homocysteine peak level after methionine loading, the treatment was changed. In intermediate response cases, vitamin B₆ therapy was continued, reinforced with folic acid 5 mg daily; in poor or nonresponse cases, vitamin B₆ was substituted by folic acid 5 mg daily. If normalization of the hyperhomocysteinemia was shown by the methionine loading test performed 6 weeks after beginning the folic acid, no further change of treatment followed. In cases in which hyperhomocysteinemia persisted (intermediate or poor or nonresponse), betaine anhydrous (Sigma 2629) 6 g daily was prescribed; folic acid was continued only if it had achieved a 60% or greater reduction. The effect of betaine was also determined by a repeated methionine loading test 6 weeks after its start. In this regimen it was thus possible for the hyperhomocysteinemic patients to have a monotherapy of either vitamin B₆, folic acid, or betaine, some combination of two of these, or even the triple combination.

Statistics

The prevalence of mild hyperhomocysteinemia in the specified categories of patients and the respective effects of treatment were analyzed by a $\chi^2$ independence test. A probability value of .05 or less was considered statistically significant.

Results

Prevalence

Mild hyperhomocysteinemia was detected in 100 of 421 vascular disease patients (24%), ie, 29 of 203 men (14%), 62 of 183 premenopausal women (34%), and 9 of 35 postmenopausal women (26%) (Fig 2). The prevalence of mild hyperhomocysteinemia in premenopausal women was significantly higher than that in male patients ($P<.0001$) both in the peripheral subgroup ($P<.002$) and in the cerebral occlusive arterial disease subgroup ($P<.002$). The prevalence of mild hyperhomocysteinemia in all patients with peripheral occlusive arterial disease (33%) was significantly higher than that in patients with cerebral occlusive arterial disease (20%) ($P<.03$).

Effect of Homocysteine-Lowering Treatment

After vitamin B₆ treatment for 6 weeks, in which the blood vitamin B₆ concentration in all 82 treated patients increased at least fivefold compared with pretreatment concentrations, the homocysteine peak level after methionine loading had normalized in 46 patients (56%), an intermediate response was found in 16 patients (20%), and in 20 patients (24%) the response was poor or absent (Figs 3 and 4). No significant difference in terms of normalization was detected between the subgroups of patients with peripheral and cerebral occlusive arterial disease (58% and 55%, respectively; $P>.8$). Vitamin B₆ treatment normalized the hyperhomocysteinemia in men significantly more frequently than in premenopausal women (71% versus 45%; $P<.05$). In terms of absolute reduction, the mean peak homocysteine concentration decreased after treatment from 30.5±8.5 to 17.7±7.0 μmol/L (mean±1 SD) in male, from 28.5±14.2 to 17.0±7.2 μmol/L in premenopausal, and from 43.7±5.5 to 31.1±22.7 μmol/L in postmenopausal patients.

Nine patients presenting an intermediate response and 13 patients presenting a poor or nonresponse


FIG 3. Plots showing response of the free homocysteine levels after methionine loading with a 6-week treatment of vitamin B6 250 mg daily in 82 hyperhomocysteinemic vascular disease patients.

FIG 4. Pie chart showing individual free homocysteine levels after methionine loading before and after a 6-week treatment of vitamin B6 250 mg daily (n=82).

Discussion

The emphasis on hyperhomocysteinemia as a risk factor for premature arteriosclerosis and thromboembolism is based on observations of patients suffering from classic homocystinuria.1-3 From a collaborative study of more than 600 homozygotes for homocystinuria, Mudd et al4 concluded that there was a 50% chance for untreated patients to have a vascular accident before the age of 30 years.4 Homocysteine, a sulfhydryl amino acid formed by demethylation of methionine, is generally considered to be an atherogenic and thrombotic agent, although the pathogenic mechanism has not been clarified.1,28-34

Mild hyperhomocysteinemia, with levels comparable to those in heterozygotes for homocystinuria, was detected by us previously* in 28% of patients with premature peripheral or cerebral occlusive arterial disease without known risk factors such as diabetes, hyperlipoproteinemia, or nonrenovascular hypertension. However, the number of patients, 25 in each group, was rather small. Since then, screening by a standardized methionine loading test of 421 such patients disclosed mild hyperhomocysteinemia in 33% of those with peripheral and in 20% of those with cerebral occlusive arterial disease. This is well in line both with our own earlier finding and with subsequent findings.6-21,35 Clarke et al,17 using a methionine loading test similar to ours, found mild hyperhomocysteinemia in up to 28% of 21 patients with peripheral and up to 42% of 38 patients with cerebral occlusive arterial disease. Patients with classic risk factors were not excluded in that study, but statistical analysis showed that mild hyperhomocysteinemia was an independent risk factor in addition to hypercholesterolemia, hypertension, and smoking.

Remarkably, mild hyperhomocysteinemia was found to be significantly more prevalent among premenopausal female compared with male vascular disease patients. This finding confirms our earlier observation6 and was reconfirmed by Brattström et al12 after screening 72 patients. We do not have a well-founded expla-
nation for this sex difference in prevalence of mild hyperhomocysteinemia. We have speculated that lower levels of serum free homocysteine in premenopausal women compared with those in men may be a sign of more efficacious methionine handling, pointing to a defense mechanism that protects women during their reproductive years against vascular disease. Failing such protection, as in cases of mild hyperhomocysteinemia, young women seem more susceptible to developing vascular disease than men. The hormonal background also may act protectively with respect to young women’s conventional risk factors. To develop arteriosclerotic events they simply may need some extra and unconventional interference such as hyperhomocysteinemia.

Now that mild hyperhomocysteinemia is generally recognized as one of the risk factors for the development of vascular disease, research into homocysteine-lowering treatment is needed. Homozygotes for homocystinuria, in whom serum free homocysteine levels in the fasting state may range as high as 200 to 300 μmol/L, respond in about 50% of cases to vitamin B6 treatment in very high doses (up to 1 g daily). Although such treatment has been found to normalize the elevated homocysteine levels in the fasting state, it does not enhance the patient’s capacity to efficiently handle a major methionine load. Treatment with folic acid, betaine, or dietary methionine restriction results in a reduction of homocysteine levels in the majority of the patients who respond poorly or not at all to vitamin B6 treatment. A significantly reduced number of initial vascular events following reduction of pathologically high homocysteine levels has been shown retrospectively in a large group of homozygotes for homocystinuria.

The present study retrospectively reviewed normalization of mildly elevated homocysteine levels after methionine intake in a large group of vascular disease patients with vitamin B6, folic acid, and betaine treatment. Brattström et al report a 26% reduction of mildly elevated homocysteine levels after methionine loading in 20 vascular disease patients after 2 weeks of vitamin B6 treatment (240 mg daily). After 2 more weeks of treatment with vitamin B6 240 mg daily plus folic acid 10 mg daily, a total reduction of elevated homocysteine levels of 39% was achieved. However, normalization of methionine handling by such treatment was not mentioned. In the present study, vitamin B6 was the first choice of treatment, and normalization of the homocysteine peak level after methionine loading was achieved in 56% of the patients. Normalization was observed considerably less frequently in premenopausal patients than in male and postmenopausal patients (45%, 71%, and 88%, respectively). From this observation one might hypothesize that a different mechanism is responsible for hyperhomocysteinemia in young women. Vitamin B6, folic acid, and betaine solely or in any combination in virtually all treated patients resulted in normalization of their overresponse by homocysteine to methionine loading. In view of these findings, we have recently treated 21 newly detected hyperhomocysteinemic patients with vitamin B6 250 mg daily combined with folic acid 5 mg daily and have found all of them to present normal responses to methionine loading within 6 weeks (data not shown).

In 18 patients from the present study in whom normalization of homocysteine levels had been achieved with vitamin B6, folic acid, and/or betaine solely or in any combination, a methionine loading test performed after 1 year of continued treatment showed persistence of the normalized homocysteine levels (data not shown). Enzyme activity determinations were not performed on a routine basis in the studied patients with mild hyperhomocysteinemia. In about half of them the vitamin B6 treatment corrected the homocysteine peak level, which suggests that these patients are heterozygotes for CS deficiency. The remaining homozygotes, in whom homocysteine handling was not normalized by vitamin B6, might be affected by either a vitamin B6–nonresponding CS mutation in heterozygous form or by a defect in homocysteine remethylation. The latter may be the trimethylamine of 5-methyltetrahydrofolate reductase (Fig 1) reported by Kang et al. This defect occurred in 17% of patients with coronary artery disease, although it did not automatically lead to mild hyperhomocysteinemia in the fasting state in the patients.

Vitamin B6 at doses from 200 mg up to 6 g has sporadically been considered as a cause of sensory neuropathy. Remarkably, these side effects were not observed in patients with homocystinuria, in whom homocysteine loading tests. Brattström et al report a 26% reduction of mildly elevated homocysteine levels after methionine treatment.2 In view of these findings, we have recently treated 21 newly detected hyperhomocysteinemic patients with vitamin B6, folic acid, and betaine solely or in any combination. Normalization was observed in 56% of the patients. Normalization was observed considerably less frequently in premenopausal patients than in male and postmenopausal patients (45%, 71%, and 88%, respectively). From this observation one might hypothesize that a different mechanism is responsible for hyperhomocysteinemia in young women. Vitamin B6, folic acid, and betaine solely or in any combination in virtually all treated patients resulted in normalization of their overresponse by homocysteine to methionine loading. In view of these findings, we have recently treated 21 newly detected hyperhomocysteinemic patients with vitamin B6 250 mg daily combined with folic acid 5 mg daily and have found all of them to present normal responses to methionine loading within 6 weeks (data not shown).

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