Treatment of coronary heart disease with folic acid: is there a future?

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CORONARY HEART DISEASE (CHD) due to atheroma in the coronary arteries is a major cause of morbidity and mortality in the developed world. The recognition of atherosclerosis as an inflammatory condition and the identification of several of its modifiable risk factors have led to new treatments. Secondary prevention of CHD now includes lifestyle modification, smoking cessation, weight loss, regular exercise, and treatment with aspirin, β-blockers, statins, angiotensin-converting enzyme (ACE) inhibitors, and ω-3 fatty acids. These treatments have reduced the risk of further cardiovascular events, but there is still room for improvement. Thus the search for new modifiable risk factors continues and one relatively new potential candidate is plasma total homocysteine (tHcy).

Biochemistry of Homocysteine and Folate

The metabolism of homocysteine and folate are closely linked (Fig. 1). Homocysteine is a naturally occurring sulfur-containing amino acid derived from the dietary amino acid methionine. Methionine is converted to S-adenosylmethionine via the enzyme methionine adenosyltransferase. S-Adenosylmethionine is then demethylated to S-adenosylhomocysteine, which is the only methyl-donating pathway in humans. This pathway is essential in providing methyl groups to produce many biomolecules such as DNA, creatine, proteins, phospholipids, and neurotransmitters. S-Adenosylhomocysteine is then hydrolyzed to homocysteine in a reversible reaction, in which S-Adenosylhomocysteine formation is favored. Once formed, homocysteine can be remethylated to methionine by two different pathways: 1) via the enzyme methionine synthase, which utilizes vitamin B12 as a cofactor and 5-methyltetrahydrofolate as the methyl donor, and 2) via the enzyme betaine-homocysteine methyltransferase. Alternatively, homocysteine can be metabolized via the vitamin B6-dependent enzyme cystathionine β-synthase (CBS) to cystathionine, which is then hydrolyzed to cysteine by cystathionase, which also requires vitamin B6 as a cofactor. Cysteine is then either converted to glutathione, taurine, or sulfate, which is excreted in the urine. During periods of excess methionine intake or if the requirements for methyl groups are low, homocysteine will enter the transsulfuration pathway. During periods of low methionine intake and/or increased requirements for methyl groups, homocysteine is remethylated to methionine.

Homocysteine Hypothesis of Cardiovascular Disease

In 1969, a report linking marked elevations of homocysteine (homocystinuria) to arteriosclerosis and thromboembolism in children with different inherited abnormalities of homocysteine metabolism led to the concept of homocysteine as a mediator of vascular disease (44). In homocystinuria, it is clear that the grossly elevated tHcy concentrations (>100 μmol/l) may play a direct role in the development of vascular and thrombotic disease. In the general population, however, tHcy concentrations are much lower (<15 μmol/l), but epidemiological studies have demonstrated that a mild to moderate elevation in plasma tHcy (hyperhomocysteinemia) is associated with an increased risk of coronary heart disease (CHD) (9). Such evidence has led to the homocysteine hypothesis of vascular disease where elevated tHcy plays a causal role in its development, and by implication, a reduction in tHcy will result in a reduced risk of CHD. However, examination of all the evidence does not appear to support this hypothesis, and epidemiological evidence of a link between plasma tHcy and CHD is conflicting.

The early retrospective and cross-sectional studies have consistently shown a strong relationship with tHcy concentrations and cardiovascular events, which contrasts with the more recent long-term prospective studies (9, 13, 16). The difference in the strength of these associations between retrospective studies, cross-sectional studies, and prospective studies may be explained by the fact that data and blood samples are collected before the event in prospective studies and therefore excludes the influence of the disease process, lifestyle, and dietary habits. However, consistent associations between plasma tHcy and the risk of CHD are found only in those prospective studies where subjects have a high risk of CHD, are older in age, and are followed up for a short period (58). However, it has been argued that the reduction in risk estimate in long-term prospective studies may be due to treatment and lifestyle and dietary changes during follow-up. These factors may modify tHcy concentrations during the course of the study (regression-dilution bias) and may underestimate any true association between plasma tHcy concentrations and cardiovascular disease (CVD) risk (14). Therefore, these results indicate that elevated concentrations of plasma tHcy may only be a short-term risk factor in subjects with a high risk of CHD or a biomarker of the degree of the underlying vascular disease. Therefore, the question of whether homocysteine per se is responsible for these associations with CHD remains unanswered.

Possible Mechanisms of Homocysteine-Mediated Cardiovascular Disease

Elevations of plasma tHcy have been linked clinically with CHD, and numerous in vitro and in vivo studies have shown that elevations of homocysteine induces endothelial dysfunction or damage (12, 39, 77). It has been argued that homocysteine exerts its damaging effects on the endothelium through mechanisms involving reactive oxygen species (42). Homocysteine contains a free sulfydryl group that, when oxidized to a disulfide, produces superoxide anions and hydrogen peroxide (45, 57). This view gained support from the observation that...
the antioxidant vitamin C was effective in preventing/reversing endothelial dysfunction in subjects with hyperhomocysteinemia after an oral methionine load (10, 35, 47). In addition, antioxidants are reported to protect endothelial cells in culture from high doses of homocysteine (7). Most of the experiments described were carried out using millimolar concentrations of homocysteine, which is ~100-fold higher than those concentrations observed in subjects with hyperhomocysteinemia. The majority of these in vitro experiments used commercial preparations of homocysteine containing the nonphysiological D-enantiomer. In addition, other studies utilized free reduced homocysteine and therefore do not reflect the complex redox thiol status that exists between the different forms of homocysteine and other thiols (67). Finally, other thiol-containing compounds such as cysteine and mercaptoethanol were found to have similar effects to those seen with homocysteine (20, 34, 65).

Therefore, the clinical relevance of these studies has to be questioned. The mechanistic studies of the potential deleterious effects of homocysteine are equivocal, especially with respect to the hypothesis that homocysteine causes oxidative stress (32). The protective effect of vitamin C is not due to its free radical-scavenging properties but most likely due to stabilizing the endothelial nitric oxide (NO) synthase (eNOS) cofactor tetrahydrobiopterin (BH4) (29) and in increasing intracellular BH4 concentrations (31). Recent evidence suggests that hyperhomocysteinemia may result in the reduction in adenosine concentrations due to the reaction kinetics favoring the formation of S-adenosylhomocysteine. Adenosine exhibits several beneficial effects on the vasculature. It can induce vasodilatation and can act as an anti-inflammatory agent; it also plays a key role in the regulation of vascular cell proliferation and death (52). However, further work is needed to explore this newly proposed mechanism.

**Homocysteine as a Marker of Inflammation**

The observation that plasma tHcy is associated with CHD may alternatively be explained if it were related to another yet-undefined confounding causal factor. Recently, it has been shown that elevated plasma tHcy and low plasma folate concentrations are associated with increased concentrations of neopterin (27, 66), itself associated with atherosclerosis (56, 63, 78), indicating that plasma tHcy may be linked to inflammation and immune system activation. Indeed, elevated plasma tHcy concentrations are associated with numerous inflammatory diseases such as psoriasis (70), systemic lupus erythematosus (51), malignancies (61), dementia (79), and rheumatoid arthritis (53). Interestingly, administration of glucocorticoids to patients with rheumatoid arthritis resulted in a significant reduction in plasma tHcy and C-reactive protein concentrations (38). Furthermore, there is evidence to suggest that homocysteine is released from damaged vascular tissue after myocardial infarction (37) and stroke (41). During the repair of damaged tissue, there is an increased demand for DNA. These repair processes require the methylation of DNA, RNA, and proteins, reactions that lead to the generation of homocysteine as the end point of methylation (Fig. 1). Optimal concentrations of folates are needed as they serve as donors of 1-carbon units in the biosynthesis of the purine ring of DNA and in the production of methyl groups. In addition, there is evidence to suggest that oxidative stress resulting from immune activation may lead to the oxidation of folates, resulting in folate deficiency despite a normal dietary intake (24). Thus hyperhomocysteinemia may be a consequence of inflammation, oxidative degradation of folates, and an end product of tissue repair. It therefore may merely be a biomarker of inflammatory disease processes including atherosclerosis.

**Endothelial Function as a Surrogate for Vascular Disease**

The endothelium is a monolayer of cells lining the entire vascular tree. It plays a central role in vascular physiology with the release of many different mediators that influence vascular tone, hemostasis, and inflammation. Of the various substances released by the endothelium, NO is the most important in terms of maintenance of vascular tone and preventing atheroma. Endothelial dysfunction or damage is a key early process in atherogenesis. Several techniques exist to assess endothelial function in clinical studies. The use of noninvasive ultrasound
to measure brachial artery flow-mediated dilatation (FMD) as an indication of endothelial function has now become a widely used surrogate measure of cardiovascular risk (73). With the use of this technique, endothelial dysfunction (as characterized by impaired FMD due to reduced NO bioavailability) is associated with all the major risk factors for atheroma (15, 82) and precedes the development of clinically detectable disease (43).

Endothelial dysfunction has been shown to be a powerful prognostic indicator for future cardiovascular events in patients with CHD (28, 54, 62). Evidence of outcome benefit after improvement in endothelial function is not yet available. However, considerable circumstantial evidence supports this strategy for improving prognosis. For example, statins (21), ACE inhibitors (23), spironolactone (22), and marine ω-3 fatty acids (25) have been shown to improve endothelial function and in separate studies, to improve cardiovascular outcome (66a, 66b, 49).

Homocysteine, Folate, and Endothelial Function

In an attempt to answer the question of causality in relation to homocysteine and vascular disease, researchers have used clinical assessment of endothelial function to assess the effects of manipulating tHcy concentrations. In healthy subjects, it is possible to induce transient hyperhomocysteinemia by giving an oral methionine load. Endothelial function is markedly impaired after this intervention (6). Pretreatment with high-dose (20 mg) folic acid prevents this adverse effect (68). It is important to point out that the dose of folic acid used in these studies (5–20 mg) is far higher than the dose needed for maximal homocysteine lowering (~0.4 mg).

Several studies in CHD patients with fasting plasma tHcy concentrations in the normal range have shown that treatment with high-dose folic acid for periods of between 6 wk and 1 yr will improve endothelial function (17, 64, 80). Some of these studies demonstrated a correlation between the extent of homocysteine lowering and improvement in endothelial function (11, 64); the largest and statistically most powerful study did not, however, demonstrate this relationship (17). This latter study suggested that folic acid may have other actions in addition to homocysteine lowering that influence endothelial function. In support of this, a recent report showed that an oral dose of 5 mg folic acid given to patients with CHD produced an acute improvement in endothelial function (by 2 h) without any change in plasma tHcy or plasma free homocysteine (18). Furthermore, intra-arterial infusion of 5-methyltetrahydrofolate (5MeTHF; the biological active form of folic acid) into the brachial artery of CHD patients also resulted in improvement in endothelial function within 30 min in the absence of a change in plasma tHcy concentration (17).

High-dose folic acid therapy has also been shown to reverse endothelial dysfunction in patients with diabetes (69) and hypercholesterolemia (72) and to prevent nitrate tolerance in the arterial circulation of healthy subjects (26). In addition to the effects of folic acid on endothelial function, folic acid in conjunction with vitamins B12 and B6 resulted in the reduction in the size of carotid atheromatous plaques (48). More recently, a similar B vitamin complex prevented restenosis in patients undergoing coronary angioplasty (55). However, further studies are needed to substantiate the effect of folate and other B vitamins in reducing plaque size and restenosis.

Potential Mechanisms of Action of Folic Acid

Homocysteine lowering. Adequate folate intake is vital because folic acid is essential for methylation reactions as well as the formation and transfer of 1-carbon units to purines and pyrimidines for DNA synthesis. Folate status is the most important determinant of an individual’s plasma tHcy concentration and can be safely, effectively, and inexpensively lowered by folic acid supplements. The tHcy-lowering effect of folic acid is maximal at daily doses of ~400 μg (29a). Folate requirements are derived on the intake necessary to prevent megaloblastic anemia, and red cell folate is used as a marker of adequacy. Recently, there has been interest in identifying intakes necessary to maintain normal 1-carbon metabolism with abnormalities identified as hyperhomocysteinemia and hypomethylation of DNA (4).

The beneficial effects of folic acid on vascular endothelial function have been attributed to the reduction in plasma tHcy concentrations. This interpretation of these recent studies has widespread support. It is clear that high-dose folic acid will improve endothelial function, and the implication is that homocysteine lowering will result in a significant reduction in cardiovascular risk. However, recent observations from clinical intervention studies indicate that plasma tHcy lowering is unlikely to be the main mechanism of action for potential outcome benefit. Low-dose folic acid supplementation can lower tHcy with no measurable reduction in cardiovascular risk, and high-dose folic acid can improve endothelial function before any change in plasma tHcy level (18). These observations suggest that high-dose folic acid has other “pharmacological” actions independent of its effects on plasma tHcy concentrations (Fig. 2). However, further work is needed to exclude an effect from changes in intracellular homocysteine or other metabolic intermediates after folic acid administration.

Reduction in superoxide production. The main circulating metabolite of folic acid in plasma is 5MeTHF, which, in vitro, when used in relatively high concentrations can reduce superoxide (17, 60) and reduce oxidative damage to human LDL (46) (Fig. 2). However, the relevance of 5MeTHF as an effective scavenger of reactive oxygen species in vivo must be questioned. The high concentrations of 5MeTHF used in these in vitro studies are not attainable in vivo after a dose of 5–10 mg of folic acid. In addition, the scavenging potency of 5MeTHF is ~20-fold lower than that of vitamin C (60).

Interaction with endothelial nitric oxide synthase. A more plausible mechanism is via the interaction of folic acid or 5MeTHF in the production of NO from eNOS (Fig. 2). Evidence for this is based on the results of clinical studies assessing the ameliorative effects of folic acid on endothelial function, which is an NO-mediated process. Production of NO by eNOS is dependent on optimal concentrations of its critical cofactor BH4 and its substrate L-arginine. In the face of an oxidizing environment, BH4 is oxidized to its inactive metabolite dihydrobiopterin (BH2), resulting in the electron “uncoupling” of eNOS, further production of damaging superoxide (71), and endothelial dysfunction (Fig. 2). Administered BH4 has been shown to scavenge reactive oxygen species in vitro and reverse endothelial dysfunction in patients with hypercholesterolemia (59). The exact mechanism whereby folic acid or its derivatives can interact with this pathway to increase NO bioavailability is unknown, but chemical stabilization of BH4,
regeneration of BH$_4$ from BH$_2$ and direct interaction of 5MeTHF with eNOS to mimick BH$_4$ have all been postulated.

Is Folic Acid a Nutritional Supplement or a Pharmacological Agent?

Folic acid in doses of 5–20 mg can reverse endothelial dysfunction in CHD patients. However, what remains unknown is the lowest dose of folic acid required to produce this effect. The question “at what dose does folic acid stop being a nutritional supplement to become a beneficial cardiovascular pharmacological agent?” has not yet been answered. Data from our own studies demonstrate that in healthy subjects despite optimum lowering of tHcy concentrations with 400 µg/day endothelial function does not improve (50). To date, there are no published studies on the effect of low-dose folic acid (400 µg) supplements on endothelial function in CHD patients. Furthermore, no study has assessed the effect on endothelial function of lowering plasma tHcy independently of the folate pathway. This can be achieved with betaine, and such a study would distinguish whether the improvement in endothelial function is independent of homocysteine lowering.

Implications for Folic Acid as Therapy for CHD

The introduction of the fortification of cereals and grains with folate (140 µg/100 g), mandated in 1996 by the United States Food and Drug Administration and in force by 1998, was intended to increase folate intake in women of childbearing age to reduce the risk of neural tube defects. This increase in folate intake has been associated with a 19% decrease in the rate of neural tube defects in the United States, part of which may be due to a continuing trend in the fall in risk of neural tube defects (30). In addition, after fortification, a reduction of hyperhomocysteinemia (defined as tHcy > 13 µmol/l) by ~50% has been observed in the Framingham offspring population study (33). Disappointingly, despite this significant reduction in plasma tHcy concentrations, no reduction in the incidence of cardiovascular mortality has yet been reported in the United States. However, a longer follow-up period may be needed to fully substantiate any effect of homocysteine lowering on cardiovascular mortality in the general population.

Many studies have confirmed the homocysteine-lowering effects of folic acid supplementation, but there are relatively few data on the effect of folate supplementation on CHD outcome. The observations that high-dose folic acid will improve endothelial function, reduce carotid artery plaque size, and reduce coronary artery restenosis rates after angioplasty are of significant potential importance, given that folic acid is a safe, cheap, and well-tolerated treatment.

In homocystinuria, due to a deficiency in the enzyme CblS, folic acid and vitamin B$_6$ lower the grossly elevated tHcy concentrations (>100 µmol/l) observed in these patients and significantly reduce cardiovascular events. This benefit is observed despite residual tHcy concentrations being several times the upper limit of “normal” (~15 µmol/l), further suggesting
beneficial actions of B vitamins independent of homocysteine lowering (81).

There are currently several large-scale randomized controlled clinical trials underway to assess the beneficial effects of folic acid and other B vitamins on cardiovascular outcome (19). These studies are designed to test the “homocysteine hypothesis” of vascular disease, and this is reflected in the dose of folic acid used. Most of the trials underway at the moment are using moderate doses of folic acid in the range of 0.2–2.5 mg/day. Hence, if folic acid has other direct pharmacological actions other than homocysteine lowering, these effects may be lost because the dose of folic acid is too low. The recently proposed Cardiac “Poly Pill” being advocated as a panacea for the treatment of CVD includes folic acid at a dose of 800 μg as one of the ingredients (76). This dose was selected as the minimum needed to ensure maximum plasma tHcy lowering (74) and the premise that reducing homocysteine will prevent CVD (75). This view should be taken with caution, because there are dangers in concluding causality from a meta-analysis of observational data. Results from the United Kingdom’s Cambridge Heart Antioxidant Study (5), a secondary intervention trial of high-dose folic acid (5 mg/day) on cardiovascular events in patients with ischemic heart disease, have recently been reported. This study comprised 942 patients on folic acid and 940 patients on placebo, with a median follow-up of 1.7 yr. Treatment significantly reduced plasma tHcy (from 11.2 ± 6.9 to 9.7 ± 5.3 μmol/l), and a twofold reduction in nonfatal myocardial infarction was observed. However, no reduction in total deaths was seen, possibly reflecting the small cohort of patients used and the short study duration. In contrast, the Goes study (40), a secondary intervention trial comprising 593 patients with CHD (300 received folic acid and 293 controls) recently reported no clinical benefit after a 2-yr intervention with low-dose folic acid (0.5 mg/day), even though plasma tHcy concentrations were significantly lowered from 12.0 ± 4.83 to 9.4 ± 3.5 μmol/l. This study was underpowered and the dose of folic acid used may be too low to demonstrate any beneficial effect other than plasma tHcy lowering. It is also important to mention that three large randomized controlled studies assessing the effect folic acid on outcomes will also be underpowered. Recruitment for the Vitamin Intervention for Stroke Prevention trial, the Women’s Antioxidant Cardiovascular Disease Study, and the HEART Outcomes Prevention Evaluation were initiated before the introduction of the fortification of cereals and grains with folic acid by the United States and Canadian governments. As a result, these trials will be substantially underpowered to test the hypothesis for which they were designed (8). For these reasons, it is important to urge caution when interpreting results from these studies (19). Further studies of longer duration and inclusion of larger sample sizes with the appropriate dose of folic acid and with all vascular events being recorded will be needed to clarify these results.

Safety Implications With Widespread Use of Pharmacological Doses of Folic Acid

It should be noted that the pharmacological doses of folic acid used in the majority of clinical studies far exceed the nutritional requirement for its normal physiological function. One issue of the potential adverse effects of fortification with or the administration of large doses of folic acid is that of masking the hematological effects of vitamin B₁₂ deficiency, thereby allowing the progression of neurological damage. The neuropathy, if untreated, may not be reversible by subsequent treatment with vitamin B₁₂. Therefore, testing for B₁₂ deficiency, or supplementation with vitamin B₁₂, may be advisable to avoid such complications. Another issue of possible concern is the appearance of high circulating concentrations of unmetabolized folic acid after the ingestion of large doses (36). The long-term effects of exposure to high doses of the synthetic form of folic acid are unknown, although as yet there is no evidence of toxicity.

Conclusions

The view that the raised plasma tHcy level is causal in the development of vascular disease is an attractive hypothesis if only because folic acid offers an easy, inexpensive, and generally safe means of lowering it. This review challenges the hypothesis that tHcy is causal and raises the possibility that an increased tHcy is an epiphenomenon. Moreover, there is evidence that the beneficial vascular effects with folic acid are only achieved in pharmacological doses. Low-dose folic acid will reduce plasma tHcy, but a high dose may be required to produce the beneficial effects on vascular function, which occur before, and apparently independently of, homocysteine lowering. The current clinical trials are on the whole designed to test the homocysteine hypothesis of vascular disease using relatively low doses of folic acid. While these trials will undoubtedly show that folic acid lowers tHcy effectively, it is unlikely that the expected reduction in cardiovascular events will be seen. However, it is important therefore not to discount treatment with folic acid if these trials are negative, because it is possible that high-dose folic acid may have a beneficial effect on outcome via mechanisms independent of homocysteine lowering. Elucidation of these mechanisms is important in the drive to develop effective treatments for prevention of CHD.

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