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Invited Review

Mechanisms and potential therapeutic targets for folic acid in cardiovascular disease

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Moens AL, Vrints CJ, Claeys MJ, Timmermans JP, Champion HC, Kass DA. Mechanisms and potential therapeutic targets for folic acid in cardiovascular disease. *Am J Physiol Heart Circ Physiol* 294: H1971–H1977, 2008. First published March 28, 2008; doi:10.1152/ajpheart.91503.2007.—Folic acid (FA) is a member of the B-vitamin family with cardiovascular roles in homocysteine regulation and endothelial nitric oxide synthase (eNOS) activity. Its interaction with eNOS is thought to be due to the enhancement of tetrahydrobiopterin bioavailability, helping maintain eNOS in its coupled state to favor the generation of nitric oxide rather than oxygen free radicals. FA also plays a role in the prevention of several cardiac and noncardiac malformations, has potent direct antioxidant and antithrombotic effects, and can interfere with the production of the endothelial-derived hyperpolarizing factor. These multiple mechanisms of action have led to studies regarding the therapeutic potential of FA in cardiovascular disease. To date, studies have demonstrated that FA ameliorates endothelial dysfunction and nitrate tolerance and can improve pathological features of atherosclerosis. These effects appear to be homocysteine independent but rather related to their role in eNOS function. Given the growing evidence that nitric oxide synthase uncoupling plays a major role in many cardiovascular disorders, the potential of exogenous FA as an inexpensive and safe oral therapy is intriguing and is stimulating ongoing investigations.

endothelial nitric oxide synthase; uncoupling; superoxide; tetrahydrobiopterin; homocysteine

THE FIRST THERAPEUTIC USE of folic acid (FA) dates back to 1931, when Lucy Wills discovered that yeast extract was effective against tropical macrocytic anemia (97) and that the critical factor involved was FA. The main role of FA was found to be its involvement in the production and maintenance of new cells (39) because it has an essential role in the integrity and function of DNA. DNA synthesis and cell proliferation require the transfer of carbon groups, a task principally fulfilled by folates. As a consequence, FA deficiency leads to inadequate nucleic acid synthesis and impairs cell division. During pregnancy, this can lead to neural tube defects, such as spina bifida; orofacial cleft; and congenital heart defects (6, 20). The risk of these developmental abnormalities is significantly reduced by FA supplementation preceding conception and during pregnancy (77). FA deficiency is also associated with the development of neoplastic and preneoplastic conditions (17), neuropathy (56) and depression (1).

FA is a water-soluble B vitamin that derives its name from the Latin word for leaf (folium) because it was first isolated from spinach leaves. Humans are unable to synthesize folate de novo and thus rely on dietary intake to derive sufficient levels of the vitamin. Rich sources include citrus fruits and juices, dark green leafy vegetables such as spinach, wheat and other whole grains, and liver. The synthetic form of FA is usually designated as folate. 5-Methyltetrahydrofolate (5-MTHF), the active metabolite, has a structure very similar to that of tetrahydrobiopterin (BH_4), an essential cofactor of endothelial nitric oxide (NO) synthase (eNOS), with the exception of an extended tail attached to 5-MTHF. It is also the primary form of folate entering the human circulation from the intestinal cells. The conversion of FA to 5-MTHF has limited capacity, however, and if enough FA is consumed orally, unaltered FA appears in the circulation (42), is taken up by cells, and is then reduced by dihydrofolate reductase to tetrahydrofolate. Over the past five years, the potential benefits of FA in the treatment of cardiovascular pathology have been revealed and have stimulated further clinical and experimental research. In this review, we discuss the potential mechanisms of action of FA and its role in the pathogenesis and treatment of different cardiovascular pathologies.

Mechanisms of Action of FA

FA is required for the remethylation of homocysteine to methionine, which in turn reduces the concentration of homocysteine available to support oxidative stress (52). FA decreases plasma homocysteine levels of both normo- and hyperhomocysteinemic subjects (61). However, FA also conveys protective effects in the absence of hyperhomocysteinemia by multiple mechanisms (Fig. 1). In the presence of sufficient cofactor BH_4, the enzyme eNOS principally synthesizes NO.

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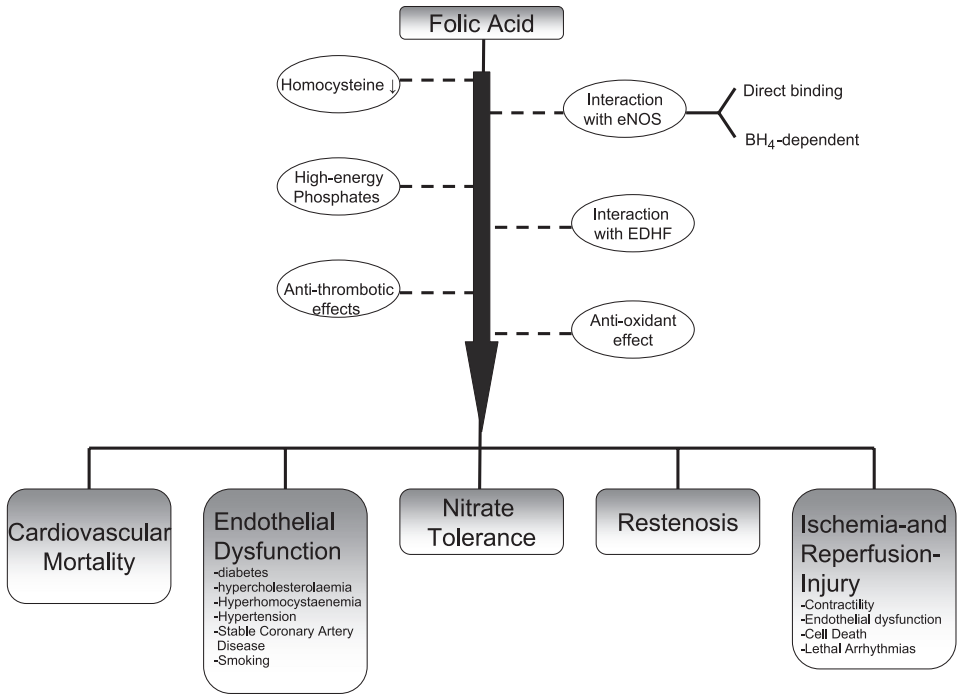

Fig. 1. Different mechanisms of action and targets of folic acid in cardiovascular diseases. eNOS, endothelial nitric oxide (NO) synthase; EDHF, endothelium-derived hyperpolarizing factor; BH_4, tetrahydrobiopterin.

Oxidative stress can oxidize BH_4 to its inactive form BH_2. Diminished bioavailability of BH_4 leads to eNOS uncoupling with subsequent decreased NO formation and increased generation of reactive oxygen species (19, 35, 45, 90). Folate can help to restore the bioavailability of BH_4 by several mechanisms. MTHF can increase the effectiveness of BH_4 on eNOS uncoupling. In theory, this can be explained by improved redox state or enhanced binding affinity of BH_4 to eNOS (improved occupancy of eNOS by available BH_4) or that MTHF facilitates the one-electron oxidation of BH_4 to the BH_4 radical (78). In addition, folate can enhance the regeneration of BH_4 from the inactive form BH_2 (41) and can chemically stabilize BH_4 (Fig. 2). Hyndman et al. (38) also found that 5-MTHF is capable of binding the pterin site in eNOS and may directly interact with eNOS independent of BH_4, although details of this interaction remain scant.

There is also evidence that FA exerts direct and indirect antioxidant effects, such as the improvement of the cellular antioxidant defense system (24, 36, 92). FA deficiency in rats increases lipid peroxidation and decreases cellular antioxidant defenses (24, 36). In healthy human volunteers, the beneficial effect of folates on postprandial endothelial dysfunction corresponded with decreased urinary excretion of malondialdehyde, a radical-damage end product (98). The administration of FA to smokers induced a significant reduction in plasma fibrinogen and D-dimer levels, markers of a prothrombotic state, and changes in plasma homocysteine did not correlate with these levels (54), suggesting an antithrombotic effect independent of homocysteine modulation. Other studies have shown antithrombotic effects of FA that are linked to a decline in homocysteine, which itself is prothrombotic (37, 47, 100) by its inhibition of a number of pathways, including thrombo-

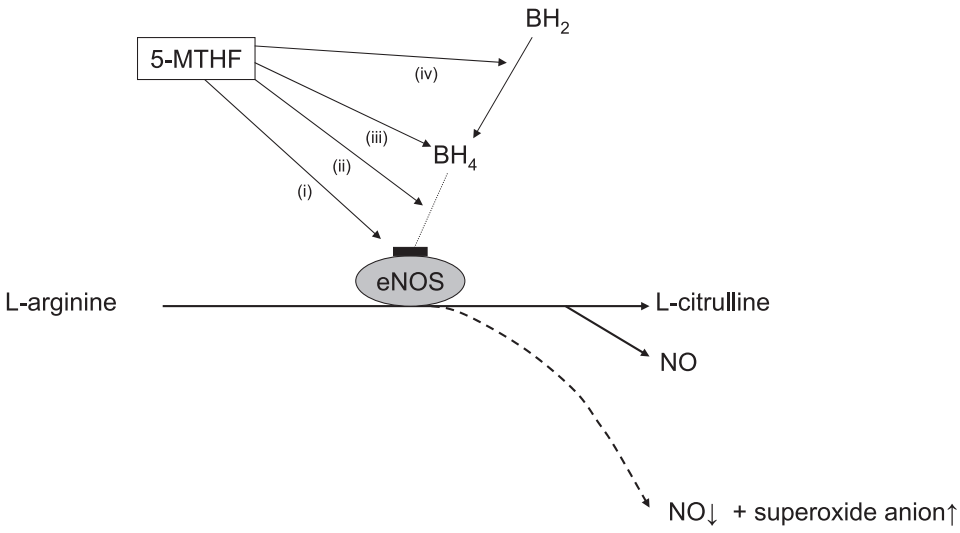

Fig. 2. Interaction of folic acid with eNOS. 5-Methyltetrahydrofolate (5-MTHF) is capable of directly interacting with eNOS (i). Folic acid also restores the bioavailability of BH_4 by ameliorating the binding affinity of BH_4 to eNOS (ii), by chemically stabilizing BH_4 (iii), and by enhancing the regeneration of BH_4 from the inactive form BH_2 (iv). Oxidative stress-induced BH_4 depletion leads to an imbalance between NO production and the generation of free radicals.

modulin expression (50), antithrombin III-binding activity of heparan sulfate proteoglycan (34), and ecto-adenosine diphosphatase activity (34). Homocysteine also stimulates endothelial plasminogen activator inhibitor-1 expression (58) and monocyte tissue factor expression and potentiates platelet aggregation (23, 43).

There are several other mechanisms that may underlie the beneficial effect of FA, but these are presently less well defined. First, 5-MTHF has been reported to restore the defective production of an unidentified endothelium-derived hyperpolarizing factor in diabetic rats (21). Second, we recently demonstrated an improved myocardial high-energy phosphate metabolism from high-dose FA pretreatment in rats subjected to regional coronary occlusion (60). As a result, less superoxide was generated, eNOS was kept in his coupled state, myocardial function was preserved, and reperfusion injury was prevented.

FA and Congenital Heart Disease

Congenital heart defects occur in ~3–8 of every 1,000 births. In the United States alone, the number of deaths attributed to congenital heart defects is estimated to be ~6,000 annually (9). The etiology of nonsyndromic congenital heart defects is complex, involving genetic, epigenetic, and environmental risk factors. However, one of the most promising clues about the prevention of conotruncal defects (truncus arteriosus, transposition of great arteries, and tetralogy of Fallot) is that women who use FA-containing vitamins in early pregnancy have reduced risks of delivering offspring with conotruncal defects (10, 75). FA is well known for its beneficial effects on neural tube closure which depend on NO synthase (NOS) activity (64). Indeed, blocking NOS activity by inhibiting BH_4 or calcium-calmodulin binding to NOS, results in ablated closure of the neural tube. Therefore, it is recommended that all women capable of becoming pregnant take 400 μg/day of FA in addition to a healthy diet (6). Women taking medications that interfere with folate metabolism (e.g., antiepileptic drugs such as carbamazepine and valproate) are advised to take higher doses of FA (1–5 mg/day) during preconception and throughout pregnancy. Furthermore, a recent study showed that the offspring of pregnant rats on a protein-restricted diet during pregnancy had higher systolic blood pressure, impaired acetylcholine-induced vasodilation, and reduced levels of eNOS mRNA in their thoracic aorta. Maternal folate supplementation during pregnancy in this model normalized blood pressure while having a modest effect on vascular function (84). These data provide a good example of how vitamin supplementation can ameliorate the adverse effects of micronutrient imbalance during pregnancy.

FA and Homocysteine

The best-known beneficial action of FA is its homocysteine-lowering effect. Homocysteine is a sulfur-containing amino acid generated during the catabolism of methionine. Homocysteine is metabolized by two pathways, either remethylation in the case of insufficient methionine or transsulfuration in the case of excess methionine. In the remethylation pathway, homocysteine is reconverted to methionine by methionine synthase, requiring vitamin B_{12} as a cofactor and FA as a methyl donor (26). With the use of the transsulfuration pathway, homocysteine is catabolized by cystathionine β-synthase, with vitamin B_6 as a cofactor, to cystathionine and subsequently to cysteine, which is excreted in the urine or incorporated into glutathionine. Hyperhomocysteinemia is therefore associated with low concentrations of methionine, FA, or vitamin B_{12}.

As a consequence of the involvement of FA in the homocysteine pathway, the oral administration (0.5–5 mg/day) results in a 25–30% reduction in the fasting homocysteine concentration (25, 33, 37a, 68, 87). No difference in the homocysteine-lowering effects of supplementary FA has been found with daily intake ranging 0.4–5 mg (37a, 87), except in patients with chronic renal failure who require a higher dose. Supplementation with vitamin B_{12} (0.02–1 mg daily) produces an additional 7% reduction in homocysteine levels and simultaneously eliminates the theoretical risk of precipitating subacute combined degeneration of the spinal cord. Vitamin B_6 supplementation has no additional effect on fasting homocysteine levels but does significantly lower postmethionine load homocysteine and cystathionine concentrations (68). Hyperhomocysteinemia, found in up to 40% of individuals with cerebrovascular, coronary, or peripheral vascular diseases (18), can be considered an independent cardiovascular risk factor (11, 76). However, the strength of association of homocysteine with the risk of cardiovascular disease may be weaker than previously believed. An updated meta-analysis of several large-scaled observational studies found that a decline in blood homocysteine of 25% (~3 μmol/l) was associated with ~11% lower risk of coronary heart disease and 19% lower risk of stroke (37b). However, most large prospective studies were underpowered for this level of risk reduction, and even larger studies are needed to prove or disprove risk modulation (3, 53).

FA and Endothelial Dysfunction

Endothelial dysfunction is a major marker of cardiovascular risk (69, 79, 94) and is characterized by reduced production/availability of NO and/or an imbalance between endothelium-derived relaxation (prostacyclin and endothelium-derived hyperpolarizing factor) and contracting (endothelin and angiotensin) factors and oxidants. A number of studies have assigned a pivotal role to oxygen-derived free radicals in accelerating NO degradation. These oxygen-derived free radicals, in particular superoxide anion, easily react with NO, decreasing its half-life.

The prevention or amelioration of coronary vascular endothelial dysfunction is an attractive goal for therapeutic interventions aimed at reducing symptoms or clinical events. In the past few years, studies have reported improved endothelial function after FA supplementation in patients with hyperhomocysteinemia (8, 14, 15, 83, 88, 99), and normohomocysteinemic patients with familial hypercholesterolemia (92, 93), diabetes (89), stable coronary artery disease (22, 59), and in smokers (55, 66). Chronic FA treatment for 6 wk in subjects with an acute myocardial infarction resulted in improved endothelial function (61). Nitroglycerin and other nitrates are the mainstay therapies for coronary artery disease but can be associated with oxygen-free radical-induced nitrate tolerance and subsequent endothelial dysfunction (81). The development of tolerance during continuous therapy is a major factor limiting the efficacy of these drugs. Supplemental FA may be

instrumented in preventing such tolerance and endothelial dysfunction (32).

FA and Atherosclerosis

FA may have beneficial effects on atherosclerosis. Carnicer et al. (13). demonstrated in apolipoprotein E-deficient mice that FA led to a decline in atherosclerotic lesions associated with increased apolipoprotein AI, AIV, and B levels and decreased oxidative stress. This was independent of plasma homocysteine and cholesterol levels. Clinical studies have employed more mixed cocktails of B vitamins. For example, a 1-yr daily B-vitamin supplementation (2.5 mg FA, 25 mg vitamin B_6, and 0.5 mg vitamin B_{12}) reduced carotid intima-media thickness compared with placebo administration (82). In one study, a long-term FA treatment (~10 yr) at a much higher dose than currently used (40–80 mg/day) was found to lower the incidence of myocardial infarction, angina pectoris, and the requirement for nitroglycerin in patients with coronary artery disease (67). This study was not placebo controlled, however, and remains an isolated observation.

Another manifestation of vascular disease where FA may be helpful is restenosis following balloon angioplasty. Schnyder et al. (72) examined 205 patients with stable coronary artery disease treated with a combination of FA (1 mg), vitamin B_{12} (400 μg), and pyridoxine (10 mg) and found reduced restenosis rates (19.6% vs. 37.6%). The extent of restenosis was also less severe. This group also observed that patients with plasma homocysteine levels below 9 μmol/l have a 49% lower rate of coronary restenosis than those with higher levels (71). However, other studies did not confirm these results (49), potentially in part because of the greater use of vascular stents in this latter study. In the Swiss Heart Study, a reduction in restenosis with FA was most observed in vessels treated with angioplasty only (10.3% vs. 41.9%, $P < 0.001$), whereas the benefit in stented lesions did not reach statistical significance (20.6% vs. 29.9%, $P = 0.32$). Differences in the pathophysiology entailed with stent placement could underlie the difference. Since thrombotic complications from sirolimus- and paclitaxel-eluting coronary stents have recently come into focus (77), the potential use of FA may again be revisited.

FA and Hemodynamic Parameters

FA has been examined for potential effects on arterial blood pressure. Tawakol et al. (80) found that high doses of FA (30 mg) acutely reduced systolic, diastolic, and mean arterial pressure. In regions of normal coronary flow, FA did not alter myocardial blood flow or adenosine reserve, whereas in abnormal zones, FA significantly improved flow reserve (49% increase with adenosine), despite the decline in pressure. Additionally, FA increased vasodilator reserve by 83% in abnormal segments but had no effect in normal segments. In another study, low-dose FA (5 mg/day), administered for 3 wk, lowered brachial pulse pressure, without altering mean arterial pressure (96), coupled to improvement in regional artery compliance.

FA and Cardiovascular Mortality

Low-serum folate levels are associated with a high risk of fatal coronary artery disease, especially when folate levels fall below 6.8 nmol/l (=3 ng/ml) (63). This inverse relationship between folate status and atherosclerotic vascular diseases has also been demonstrated in the Nutrition Examination Survey (28, 31, 51), the Kuopio Ischemic Heart Disease Risk Factor Study (95), and the Framingham Heart Study (74), although it has not been confirmed by others: the Physicians' Health Study (16) and Atherosclerosis Risk in Communities Study (27). Antifolate therapy with methotrexate has been suggested to promote atherosclerosis (48). Beyond dietary reductions, a common mutation of 5,10-MTHF reductase caused increased thermolability and reduced activity of the enzyme catalyzing reduction of 5,10-methylenetetrahydrofolate to 5-MTHF. This mutation has been reported as a risk factor for cardiovascular disease (29, 30, 40, 46, 62).

Pharmacological Considerations of FA

In general, FA supplementation is considered safe (12), and there is no evidence that high natural folate intake poses a toxicity risk (65). No adverse effects have been reported when high doses of FA (40–80 mg/day) are administered for as long as 10 years (67). Only one study reported on the use of FA (300 mg/kg, once a week for 4 wk) in rats as a model for interstitial nephritis (86). However, converting this dose from a rat to a human of 75 kg results in a dose of 22.5 g. The main safety concern lies in the fact that folate can mask the diagnosis of pernicious anemia, because high FA levels correct the anemia but allow the neuropathy to progress undiagnosed to an irreversible degeneration of the spinal cord (73). Therefore, vitamin B_{12} levels should always be measured before the start of supplementation with FA. Another concern that needs special attention is the role of FA in carcinogenesis. In established neoplasms, the inhibitory and promoting effect of folate deficiency and supplementation, respectively, has been well described and has been the basis for cancer chemotherapy with several antifolate agents (e.g., methotrexate) and 5-fluorouracil. In neoplastic cells, in which DNA replication and cell division occur at an accelerated rate, the interruption of folate metabolism causes ineffective DNA synthesis, resulting in the inhibition of tumor growth (32, 33). In contrast, the role of FA, and in particular of folate fortification, on de novo carcinogenesis in normal tissue has been the subject of many contradictory reports over the past decade (44). Very recently, Bayston et al. (7) reported that there is no ground of concern to avoid the fortification with FA and that FA supplementation will not enhance the risk on colorectal carcinomas.

The synthetic form of FA (folate) is used in supplements and is added to food because of its high stability and bioavailability. The metabolic active form of FA, 5-MTHF, is also readily available. Unlike FA, 5-MTHF has to be converted to tetrahydrofolate via the vitamin B_{12}-dependent enzyme methionine synthase. In case of vitamin B12 deficiency, 5-MTHF is not converted to tetrahydrofolate and thus is not able to improve megaloblastic anemia, even when given at high doses. Furthermore, 5-MTHF does not require a reduction by dihydrofolate reductase to be incorporated into the active cellular folate pool (101). However, low-dose 5-MTHF is equally effective since FA in reducing homocysteine concentrations in healthy persons (91) and restoration of endothelial function can also be performed by an infusion of 5-MTHF (92).

Uremic patients usually have elevated levels of homocysteine and are relatively resistant to FA therapy. The reason for

this phenomenon is unknown but may be due to impaired intestinal absorption and/or impaired metabolic transformation of FA to an active form (57). Folinic acid (5-formyltetrahydrofolate) supplementation to this population may be more efficient in reducing the high homocysteine level in uremia (2, 57, 85). Folinic acid can be given intravenously, where it normally is readily converted (via 5,10-methenyltetrahydrofolate and 5,10-methylenetetrahydrofolate) to 5-MTHF (70). This form of FA is best known for counteracting the therapeutic and toxic effects of FA antagonists, such as methothrexate, in the treatment of tumors, rheumatoid arthritis, and psoriasis.

Conclusion

Coronary artery disease has become the leading cause of death in Western countries. Various studies have demonstrated an association between low-serum folate levels and the risk of fatal coronary artery disease. In light of this observation, FA not only appears important for risk stratification but also opens new therapeutic possibilities in the treatment of cardiovascular diseases. Apart from various promising results on eNOS-dependent superoxide generation in animal studies and its well-known homocysteine-lowering effect, FA can benefit on endothelial dysfunction, and recent work suggests a potential to preserve myocardial function and prevent tissue damage.

Some of these effects may require high doses of FA, much higher than those tested to date, and clearly much higher than those obtainable through the diet. Precisely when and why higher doses might be required for some therapeutic targets remain unclear and somewhat controversial. Clearly, additional studies are needed to further clarify the potential role of FA, not only for risk stratification but also for cardiovascular disease treatment and/or prevention.

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