Editorial

Homocysteine Lowering: Any Use in Atherosclerosis?

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Plasma levels of the amino acid homocysteine have been associated with the risk of coronary atherosclerosis in the general population.[1] In addition, increased homocysteine levels have a prognostic value in patients with established coronary artery disease, and plasma total homocysteine has been identified as a strong predictor of mortality in patients with angiographically confirmed coronary artery disease.[2] The "normal" range of total homocysteine levels is between 5-15 μmol/L (mean level 10 μmol/L) in healthy individuals, while homocysteinaemia has been defined as plasma total homocysteine levels >15 μmol/L.[3] Homocysteinaemia has been stratified as mild (15-29 μmol/L), moderate (30-99 μmol/L) and severe (≥100 μmol/L), with moderate and severe forms being strongly associated with a prothrombotic state.[1] Homocysteine levels are determined by complex interactions between environmental factors, lifestyle parameters and genetic background. The main strategy to reduce homocysteine levels is treatment with folic acid and B-vitamins.[1]

Since the first epidemiological observations, a large number of studies have investigated the mechanisms relating homocysteine to cardiovascular risk. For example, homocysteine has been demonstrated to induce oxidative stress in experimental models[4] and in humans.[5] This is achieved by several mechanisms, such as activation of NADPH-oxidases,[6] uncoupling of endothelial nitric oxide synthase (eNOS),[7] down-regulation of intracellular antioxidant defense systems,[8] and many others. The generation of reactive oxygen species (ROS) decreases endothelial nitric oxide (NO) bioavailability and leads to oxidative damage in the vascular wall, thus contributing to atherogenesis.

Homocysteine lowering is a potential therapeutic approach to prevent and treat atherosclerosis. Indeed, several studies have demonstrated that lowering homocysteine with folates and B-vitamins exerts a global beneficial effect on vascular function by improving endothelium-dependent vasodilation,[9] aortic distensibility,[9] and intima-media thickness[10] in humans, all important measures with a predictive role in atherosclerosis. However, it has also been suggested that folic acid may have additional benefits for vascular function, via mechanisms independent of homocysteine lowering.[11] We have recently shown that 5-methyl-tetrahydrofolate (5-MTHF), the circulating form of folic acid, can act as a scavenger of peroxynitrite radicals and increases intracellular levels of eNOS cofactor tetrahydrobiopterin (BH4) in human vascular endothelium.[12] The elevation of intracellular BH4 leads to an improvement of eNOS coupling that increas-

es NO bioavailability and decreases eNOS-derived superoxide generation in endothelial cells.[12] Therefore, treatment with folic acid or 5-MTHF improves vascular redox state and may modify intracellular redox signaling in human vascular endothelium.

Although epidemiological studies, basic research and clinical science suggest that homocysteine is a modifiable risk factor for atherosclerosis, the potential of homocysteine lowering treatment with folic acid to reduce cardiovascular risk remains uncertain and controversial. Following the introduction of folate fortification of flour in North America a considerable reduction of cardiovascular risk (and especially stroke) was observed, compared to the European population, where folate fortification was not introduced.[13] This observation supported the initial hypothesis that lowering homocysteine may have a beneficial effect on cardiovascular risk, but these findings had to be confirmed by randomised, prospective clinical trials.

During the last 2 years, the results of two large randomised clinical trials examining the long-term effect of folate/B-vitamins treatment on cardiovascular risk were published. The NORVIT study[14] examined the effect of folic acid (800 μg/d) and vitamins B6 (40 mg/d) and B12 (400 μg/d) on the clinical outcome of 3749 patients with recent myocardial infarction, over a period of 3.5 years. The NORVIT study demonstrated that homocysteine-lowering treatment had no effect on survival in these patients. However, this study had a number of confounding factors, which have to be taken into account when interpreting its results. First, the population consisted of patients with recent myocardial infarction, and these patients also began treatment with statins, angiotensin converting enzyme inhibitors, and beta-blockers at the same time. In addition, the study was powered to detect >18% reduction of cardiovascular risk over 3.5 years, whereas most of the events occurred during the first year of follow-up, as a consequence of myocardial infarction.

The results of the HOPE-2 study[15] were also not as clear as was hoped. In the HOPE-2 study, the effect of homocysteine-lowering treatment with folic acid (2.5 mg/d), vitamin B6 (50 mg/d) and B12 (1 mg/d), on cardiovascular risk was evaluated in 5522 patients with vascular disease or diabetes over a period of 5 years. Although the study demonstrated a significant reduction of the risk for stroke, it failed to demonstrate any effect on either overall cardiovascular mortality or combined cardiovascular risk. Importantly, 70% of the population in the HOPE-2 study were recruited in areas with dietary folate fortification, and self-treatment with dietary vitamin supplements was not excluded, so that folate levels at baseline were not low. In this folate pre-treated population the statistical power to demonstrate an effect of folate treatment would be greatly diminished. Indeed, a non-significant trend towards the reduction of cardiovascular risk was observed in the subgroup of patients from non-fortified areas and requires further investigation.

How can we interpret these conflicting data between basic research/clinical science and randomised clinical trials? If homocysteine is a risk factor for atherosclerosis and folate has direct beneficial effects on vascular function, then why does folate treatment fail to improve clinical outcome in patients with atherosclerosis? One possible mechanism is that, through its role in the synthesis of thymidine, folic acid promotes cell proliferation leading to a worsening of atherosclerosis.[16] Another possible mechanism is based on the relation of homocysteine to the methylation cycle: folate induces the remethylation of homocysteine to methionine, which in turn lowers *S*-adenosylhomocysteine and increases *S*-adenosylmethionine levels. This latter molecule is a main source of methyl groups for all methylation reactions in the cell, which include the intracellular methylation of L-arginine to asymmetrical dimethylarginine or the local hypermethylation and hypomethylation of genes with a key role in atherogenesis. A third explanation for the negative results of the HOPE-2 study has emerged from a study of the behaviour of 5-MTHF in human vascular wall. Indeed, we observed that treatment of patients with coronary atherosclerosis, using a folic acid dose similar to what people receive as folate fortification (recommended daily allowance 400 μg/d), improved endothelial function and arterial distensibility, reduced vascular superoxide generation and improved eNOS coupling by improving the bioavailability of BH4 in human vascular endothelium.[9] Importantly, we observed that treatment with a higher dosage of folic acid (5 mg/d) induced a similar improvement in these parameters of vascular function, despite the significantly higher levels of 5-MTHF. That observation was explained by the fact that both high- and low-dose treatment with folic acid induced a similar elevation of 5-MTHF in the vascular wall, despite the higher plasma levels of 5-MTHF in the high-dose treatment group. This finding is compatible with the hypothesis that 5-MTHF does not enter endothelial

cells by diffusion, but is probably taken up by the cells, depending on the cellular demands. Therefore, the recommended daily allowance for folic acid (400 μg/d) has the ability to induce the maximum benefit in terms of vascular function, and if this amount is received by food folate fortification or by a folate-enriched diet, then any further treatment may induce no further benefit. This hypothesis partly explains why the HOPE-2 study was negative, and why future studies conducted in North America may also provide negative results. This population receives the maximum benefit of folate through folate-food fortification, and any further treatment with folate on top of that is unlikely to improve vascular function, since high-dose treatment increases circulating but not intracellular 5-MTHF in these patients.

In conclusion, plasma total homocysteine is independently associated with increased cardiovascular risk. Moderate or severe homocysteinaemia is associated with thromboembolic events and increased risk for atherothrombosis, and should be treated with folic acid/B-vitamins. However, it is still unclear whether homocysteine-lowering treatment with folic acid/B-vitamins has the potential to improve the clinical outcome in subjects with mild homocysteinaemia, and the debate on whether homocysteine-lowering treatment with folate has a role in primary or secondary prevention in subjects with "normal" plasma homocysteine is still active.[17] More large-scale clinical trials evaluating the effect of low-dose folate treatment on cardiovascular risk in unfortified populations are necessary, in order to clarify whether folate food fortification has the potential to decrease cardiovascular risk in the general population.

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