Reloaded: ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inaemia: effects of L-arginine and B vitamins

AUTHORS’ RETROSPECTIVE

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Online publish-ahead-of-print 9 August 2012

This editorial refers to an article by K. Sydow et al.11 published in Cardiovascular Research in 2003 (see Box 1). It is accompanied by an editorial by J.-L. Balligand, pp. 165–166, this issue, as part of this Spotlight on Landmark Papers in Cardiovascular Research.

1. What was going on in the field at the time?

1.1 Hyperhomocysteinaemia

In 1997, when we were designing our clinical study, hyperhomocysteinemia was considered to be a potential independent cardiovascular risk factor.1,2 A variety of underlying causes, i.e. inherited enzyme defects, renal insufficiency, and acquired defects in homocysteine metabolism, had been discovered. Since B vitamins and folic acid are relevant cofactors for metabolizing homocysteine, their combined treatment had been proved to significantly lower homocysteine plasma concentrations. However, the impact of combined B vitamin treatment on cardiovascular disease had been considered controversial.

1.2 Endothelial dysfunction: role of nitric oxide and asymmetric dimethylarginine

Nitric oxide (NO) plays a crucial role in regulating vascular homoeostasis. Mechanisms that lead to endothelial dysfunction include a reduced NO production and/or an increased inactivation of NO. Asymmetric dimethylarginine (ADMA) is an endogenous NO synthase (NOS) inhibitor. In 1992, Vallance et al.3 demonstrated elevated ADMA plasma concentrations in patients with renal insufficiency and revealed that exogenous administration of ADMA resulted in endothelial dysfunction with a concomitant increase in blood pressure. By the time we initiated our clinical study, the plasma concentrations of ADMA were shown to be elevated in hypercholesterolaemic rabbits and in patients with peripheral arterial disease (PAD) and hypercholesterolaemia.4,5

2. How did we come to perform that study?

There was initial evidence that hyperhomocysteinaemia was associated with the NO pathway and endothelial dysfunction in animals and humans.6,7 Homocysteine increases the oxidative degradation of NO through the formation of disulfides and the generation of hydrogen peroxide and superoxide anion. Interestingly, ADMA plasma concentrations increased rapidly after acute methionine loading in humans.8 This methionine-induced increase in ADMA and homocysteine concentrations resulted in impaired flow-dependent vasodilation of the brachial artery. In these individuals, endothelial function inversely correlated with ADMA plasma concentration and directly with the L-arginine/ADMA ratio. In addition, monkeys fed with a methionine-rich diet showed elevated ADMA plasma concentrations.9 Intriguingly, lowering homocysteine concentrations with B vitamins in these animals did not improve endothelial function.

Therefore, we were looking for a biochemical pathway linking the homocysteine pathway with the L-arginine-NO-ADMA pathway. Going back to the literature, we found S-adenosyl-L-methionine (SAM) to be a potent methyl group donor with the ability to methylate protein-bound arginine residues. Interestingly, SAM plays a crucial role in the demethylation pathway of homocysteine.
ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins

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Received 8 May 2002; accepted 26 July 2002

Abstract

Objectives: Hyperhomocyst(e)inemia is a risk factor for atherosclerotic vascular disease, and it is associated with endothelial dysfunction. Mechanisms responsible for endothelial dysfunction in hyperhomocyst(e)inemia may involve impaired bioavailability of NO, possibly secondary to accumulation of the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) and increased oxidative stress. We investigated whether oral treatment with B vitamins or L-arginine normalizes endothelium-dependent, flow-dependent vasodilation (FDD) in patients with peripheral arterial occlusive disease (PAOD) and hyperhomocyst(e)inemia. Methods: 27 patients with PAOD and hyperhomocyst(e)inemia were assigned to oral treatment with combined B vitamins (folate, 10 mg; vitamin B12, 200 µg; vitamin B6, 20 mg/day), L-arginine (24 g/day) or placebo, for 8 weeks in a double-blind fashion. FDD was determined by high-resolution ultrasound in the radial artery. Results: Vitamin B supplementation significantly lowered plasma homocyst(e)ine concentration from 15.8±1.8 to 8.7±1.1 µmol/l (P<0.01). However, B vitamins had no significant effect on FDD (baseline, 7.8±0.7%, B vitamins, 8.3±0.9%, placebo 8.9±0.7%; P=n.s.). In contrast, L-arginine treatment did not affect homocyst(e)ine levels, but significantly improved FDD (10.2±0.2%), probably by antagonizing the impact of elevated ADMA concentration (3.8±0.3 µmol/l) and reducing the oxidative stress by lowering urinary 8-iso-prostaglandin F\textsubscript{2α} (baseline, 76.3±7.1 vs. 62.7±8.3 pmol/mmol creatinine after 8 weeks). Conclusions: Oral supplementation with combined B vitamins during 8 weeks does not improve endothelium-dependent vasodilation in PAOD patients with hyperhomocyst(e)inemia, whereas L-arginine significantly improved endothelial function in these patients. Thus, accumulation of ADMA and increased oxidative stress may underlie endothelial dysfunction under hyperhomocyst(e)inemic conditions. These findings may have importance for evaluation of homocyst(e)ine-lowering therapy.

Keywords: Atherosclerosis; Endothelial function; Nitric oxide

Box I Title page including abstract from the original 2003 publication in Cardiovascular Research.\textsuperscript{11} Used with permission of Oxford University Press on behalf of the European Society of Cardiology.

and methionine. Using an in vitro approach with cultured human endothelial cells, we were able to demonstrate that SAM indeed had the capability of transferring methyl groups to arginine, yielding ADMA.\textsuperscript{10} Intriguingly, the incubation of homocysteine or methionine significantly increased ADMA concentrations in the cell supernatant, and this homocysteine-induced increase in ADMA was even enhanced in the presence of vitamins B6, B12, and folic acid.\textsuperscript{10}

Therefore, we investigated whether the combined supplementation with vitamin B12 (200 µg cyanocobalamin/day), vitamin B6 (20 mg pyridoxal 5′-phosphate/day), and folic acid (10 mg/day) or L-arginine (24 g/day) was able to reduce moderately elevated homocysteine plasma concentrations and to improve endothelium-dependent, NO-mediated vasodilation in PAD patients in a placebo-controlled, double-blind fashion.\textsuperscript{11} Indeed, we were able to demonstrate that combined B vitamin supplementation did not improve endothelium-dependent vasodilation despite a significant reduction in homocysteine plasma concentrations. However, supplementation of the NO precursor L-arginine—which did not significantly lower homocysteine plasma concentrations—improved vascular function in these patients (Figure 1).

3. Where has the field moved since then?

3.1 Association between homocysteine and asymmetric dimethylarginine

Several case-control studies demonstrated an association between hyperhomocysteinaemia and elevated ADMA plasma concentrations in humans with coronary artery disease, PAD, and stroke.\textsuperscript{12–14} Recently, Schwedhelm et al.\textsuperscript{15} defined reference levels of ADMA concentrations using a reference sample from the Framingham Offspring Study consisting of 1126 non-smoking individuals who were free of clinical cardiovascular disease, hypertension, diabetes, and obesity. Interestingly, ADMA plasma concentrations were positively correlated with age and total plasma homocysteine following a multivariable
regression analysis in this study sample. Overall, the findings of these clinical studies provided strong evidence that ADMA contributes to NO impairment and endothelial dysfunction in hyperhomocysteinaemia.

The mechanisms by which ADMA becomes elevated in hyperhomocysteinaemia seem to be multifactorial. The activity of protein arginine \textit{N}-methyltransferase (PRMT), the enzyme responsible for the SAM-dependent transfer of methyl groups to L-arginine in the synthesis of ADMA, may be enhanced under hyperhomocysteinaemic conditions induced by a methionine load. However, hyperhomocysteinaemia results in a greater accumulation of S-adenosyl-L-homocysteine than SAM, which seems to be in contrast to our initial hypothesis. Nevertheless, despite of a lack of enhanced PRMT activity by SAM, it still remains possible that hyperhomocysteinaemia enhances PRMT expression.

In addition to increased ADMA synthesis by enhanced PRMT activity and/or expression, impaired activity, and/or expression of the enzyme that is responsible for ADMA degradation—dimethylarginine dimethylaminohydrolase (DDAH)—seems to be another significant factor for ADMA accumulation in hyperhomocysteinaemia. Interestingly, homocysteine has been shown to inhibit DDAH activity in vitro modulated by an oxidative reaction of homocysteine with the active-site cysteine residue of DDAH. In addition, S-nitrosyl-L-homocysteine powerfully inhibits DDAH activity. In vitro and in vivo approaches have revealed that hyperhomocysteinaemia causes tissue-specific decreases in DDAH expression without altering plasma ADMA concentrations in mice with endothelial dysfunction.

Mice overexpressing human DDAH1 have been generated and have been shown to be protected from adverse vasomotor effects of ADMA on endothelial function. Using this transgenic murine model, Rodionov et al. tested the hypothesis that hDDAH1 overexpression protects from hyperhomocysteinaemia-induced vascular dysfunction, vascular hypertrophy, and thrombosis. Interestingly, these mice were not protected against endothelial dysfunction and/or accelerated thrombosis, but were protected with regard to vascular muscle function and the development of cerebral vascular hypertrophy. The lack of the elevation of ADMA plasma concentrations in these hyperhomocysteinaemic mice suggests that the beneficial hDDAH1 effects may be—at least in part—-independent of ADMA.

### 3.2 Hyperhomocysteinaemia and cardiovascular disease

Hyperhomocysteinaemia appears to be an independent risk factor for cardiovascular and venous thromboembolic disease. A meta-analysis that evaluated data from 30 prospective and retrospective studies involving >5000 ischaemic and 1000 stroke events revealed a 25% lower homocysteine concentration in the prospective studies being associated with a lower risk for ischaemic heart disease. In addition, another meta-analysis demonstrated that patients with the methylene tetrahydrofolate reductase TT genotype had higher odds of coronary heart disease compared with controls. The issue of whether homocysteine plays a causal role in cardiovascular disease or whether there is a non-causal association has been addressed by several meta-analyses with varying positive or negative associations.

Factors that may contribute to the negative association in these clinical studies include publication bias and a geographical variability in folate intake. Considering the results of the clinical trials that have been conducted in the meantime, hyperhomocysteinaemia does not appear to...
be as important as other classical cardiovascular risk factors, i.e. diabetes, hypertension, hypercholesterolaemia, and smoking.

Meta-analyses of randomized clinical trials for supplementation aimed at lowering homocysteine concentrations in patients with cardiovascular disease have revealed controversial results. In the HOPE-2 trial treatment with combined B vitamins did not affect the primary combined endpoint of cardiovascular death, myocardial infarction, and stroke after a mean follow-up of 5 years, despite a 2.4 μmol/L decrease in mean homocysteine concentrations. Overall, the majority of studies found no decrease in cardiovascular events and/or death. Nevertheless, it remains possible that the study design (e.g. missing assessment for adequate nutritional conditions, trials performed in regions with high folate concentrations in the population, inclusion of individuals without increased homocysteine concentrations, etc.) may have been a significant influence for the negative results.

Therefore, although still controversial, the current recommendation may be not to screen for moderate hyperhomocysteinaemia and not to treat patients with cardiovascular disease with combined B vitamin supplementation—neither for secondary nor for primary prevention. However, results of several randomized clinical trials investigating combined B vitamin supplementation for primary prevention of cardiovascular disease considering large numbers of individuals and various dosages and combinations may affect future recommendations.

### 3.3 Asymmetric dimethylarginine and cardiovascular risk

Currently available data from prospective clinical studies in which ADMA has been determined in populations at a broad range of global vascular risk reveal that ADMA is significantly associated with an increased risk of incident cardiovascular events and mortality. In 2001, two studies revealed for the first time that elevated ADMA concentrations seem to be a predictor for all-cause and cardiovascular mortality in patients with chronic renal insufficiency and acute coronary events. Schnabel et al. investigated whether the determination of ADMA serum concentrations improves cardiovascular risk prediction, in particular in comparison with traditional risk factors and biomarkers, i.e. C-reactive protein and B-type natriuretic peptide. In this prospective cohort of 1874 consecutive patients with coronary artery disease, mean ADMA concentrations were higher among individuals who subsequently developed the primary cardiovascular endpoint. Furthermore, the risk of future cardiovascular events was associated with an increase by a third of the baseline ADMA. Therefore, high baseline ADMA serum concentrations seem to independently predict future cardiovascular risk and may add an additional prognostic value beyond classical cardiovascular risk factors. In the Gothenburg Study, any 0.15 μmol/L increase in ADMA was associated with a 30% increase in risk for fatal and non-fatal myocardial infarction, while in the Framingham Offspring cohort, any 0.13 μmol/L increase was associated with a 21% increase in risk in the overall population and with a 30% increase in risk in non-diabetic individuals.

Beyond the observational findings of various clinical studies, the generation of DDAH overexpressing and knock-out mouse models has significantly contributed to the understanding of the underlying mechanisms by which ADMA contributes to cardiovascular disease. Interestingly, ADMA seems to exert additional effects aside from simply regulating vascular tone by inhibiting NOS and decreasing NO bioavailability. Mice overexpressing a human DDAH1 transgene develop less transplant vasculopathy after heterotopic heart transplantation and show an accelerated endothelial repair after endothelial denudation.

A recent study underlined the result of these two animal models that ADMA seems to be an important modulator of inflammation in cardiovascular disease.

Overall, the results of the prospective clinical studies suggest that ADMA may be a suitable diagnostic marker for cardiovascular risk assessment, since the hazard ratios for ADMA were mostly in a range comparable with that of traditional cardiovascular risk markers even after multivariable adjustments. However, the relatively narrow distribution of ADMA plasma concentrations suggests a tight physiological and pathophysiological control of ADMA which may make it difficult to individually predict cardiovascular risk. Nevertheless, modulating biochemical pathways that regulate ADMA concentrations or that are modulated by increased ADMA concentrations (e.g. inflammatory cascades, etc.) may be a promising therapeutic approach to treat or even prevent cardiovascular disease.

### References


