REVIEW

ASYMMETRIC DIMETHYLARGININE (ADMA) AND ENDOTHELIAL DYSFUNCTION: IMPLICATIONS FOR ATHEROGENESIS

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Atherosclerotic coronary heart disease is the leading cause of morbidity and mortality in industrialized countries, and endothelial dysfunction is considered a precursor phenomenon. The nitric oxide produced by the endothelium under the action of endothelial nitric oxide synthase has important antiatherogenic functions. Its reduced bioavailabilty is the beginning of the atherosclerotic process. The addition of two methyl radicals to arginine, through the action of methyltransferase nuclear proteins, produces asymmetric dimethylarginine, which competes with L-arginine and promotes a reduction in nitric oxide formation in the vascular wall. The asymmetric dimethylarginine, which is itself considered a mediator of the vascular effects of the several risk factors for atherosclerosis, can be eliminated by renal excretion or by the enzymatic action of the dimethylarginine dimethylaminohydrolases. Several basic science and clinical research studies suggest that the increase in asymmetric dimethylarginine occurs in the context of chronic renal insufficiency, dyslipidemia, high blood pressure, diabetes mellitus, and hyperhomocysteinemy, as well as with other conditions. Therapeutic measures to combat atherosclerosis may reverse these asymmetric dimethylarginine effects or at least reduce the concentration of this chemical in the blood. Such an effect can be achieved with competitor molecules or by increasing the expression or activity of its degradation enzyme. Studies are in development to establish the true role of asymmetric dimethylarginine as a marker and mediator of atherosclerosis, with possible therapeutic applications. The main aspects of the formation and degradation of asymmetric dimethylarginine and its implication in the atherogenic process will be addressed in this article.

KEYWORDS: Coronary heart disease; Atherogenesis; Endothelium; Nitric oxide; Asymmetric dimethylarginine; Cardiovascular risk.

INTRODUCTION

Atherosclerotic coronary heart disease, which can cause an acute occlusion of one or more coronary arteries, is the leading cause of morbidity and mortality in industrialized countries. Over three million men and women die each year due to this disease[1] and, according to the World Health Organization, it will continue to be the leading cause of mortality in the world for the foreseeable future.[2]

The presence of risk factors for cardiovascular disease, mainly in the context of atherosclerotic disease, induces changes in the functional as well as in the morphologic aspects of the endothelium, readily leading to inflammation, thrombosis and vasoconstriction.[3] A dysfunctional endothelium can be detected by an imbalance between dilating and constricting factors, the procoagulant and the anticoagulant factors, and the stimulating and inhibiting activities related to cellular development and proliferation.[4]

It becomes necessary to understand the participation of the endothelium in the physiopathogenesis of this disease using epidemic characteristics. In this review article, we will try to summarize the physiological defects of the endothelium that are brought on by antagonism of the enzyme responsible for the formation of nitric oxide.

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Nitric oxide in vascular homeostasis

The first evidence that the endothelium plays a role in vascular tonus control through the production of vasoactive substances arose in 1977. [5] Later, in 1980, it was postulated that there may exist an endothelium-related relaxing vascular factor.[6] Subsequently, two independent research groups proved that this relaxing factor was nitric oxide (NO).[7,8]

NO is a free radical that, by definition, is a molecule that can exist independently and that features an isolated electron in the outermost electron layer.[9] It is a small molecule that is generated in biological tissues by specific nitric oxide synthases (NOS) by way of an oxidative reaction involving five electrons.[10] This highly reactive molecule performs an important oxidative biological signaling role in various processes.[11] It has a half-life of a few seconds in aqueous media, a higher stability in a medium with a low oxygen concentration, and is soluble in a lipid-rich environment, allowing for quick diffusion within the cytoplasm and through plasma membranes.[12,13]

NO carries out its antiatherogenic effects by stimulating the release of soluble guanylate cyclase (GC), producing cyclic guanosine monophosphate (cGMP) from cyclic guanosine triphosphate (cGTP)[5-8] (Figure 1). It originates in the endothelium via the conversion of L-arginine to L-citrulline in the presence of endothelial NOS (eNOS), which is nicotinamide adenine dinucleotide phosphate (NADPH)-dependent and requires as cofactors Ca^{+2}/calmodulin (CaM), flavin adenine dinucleotide (FAD),

flavin mononucleotide (FMN) and tetrahydrobiopterin (BH_4).[14]

The NO generated by eNOS is important to blood flow maintenance because it regulates muscular tone, influences the interaction of the white cells and platelets with the endothelium and limits the development of neointimal hyperplasia by inducing apoptosis of the smooth muscle cells of the vessel.[15] The inducible isoform of the NOS (iNOS) can produce high quantities of NO during inflammation, turning it into a cytotoxic agent due to its free radical properties.[16] The neuronal isoform (nNOS) is mainly important for the relaxation of the smooth muscle cells, but it also performs a role in behavioral inhibition.[17]

Reduced bioavailability of NO in the vascular wall can be a result of diminished expression of eNOS, the absence of its substrates or cofactors, or a change in cellular signaling. All of these may lead to improper inactivation of the enzyme and accelerated degradation of NO by reactive oxygen species (ROS) such as oxygen ions, free radicals and peroxides.[18]

ADMA and endothelial dysfunction

On account of these discoveries, the endothelium was accepted by researchers as a paracrine organ.

Biochemists first discovered that methylated arginines are excreted in the urine[19] and it has since been demonstrated that asymmetric dimethylarginine (ADMA) antagonizes endothelium-dependent vasodilation, a phenomenon first observed in chronic renal failure.[20]

ADMA is now known to be a mediator molecule of the adverse vascular effects of many other factors and markers of cardiovascular risk. It is characterized as an amino acid of intracellular origin naturally found circulating through the plasma, urine, tissues, and cells.[20] It is synthesized when arginine residues in the nuclear proteins are methylated through the action of the protein arginine methyltransferases (PRMTs), which are largely distributed throughout the human body, through a posttranslational change that adds one or two methyl groups to the nitrogens of the guanidine incorporated into the proteins[21] (Figure 2). There are two types of PRMTs, with several isoforms: type 1 catalyzes the formation of ADMA, and type 2 catalyzes the formation of the symmetric dimethylarginine (SDMA); but both enzymes can transfer the methyl radical, producing the NG-monomethyl-L-arginine (L-NMMA). Of these, only the asymmetrically methylated species (ADMA and L-NMMA) are inhibitors of NOS; SDMA is not.[21]

ADMA inhibits the three isoforms of NOS, and is equipotent with L-NMMA. It can also uncouple the enzyme, generate superoxides, and it interfaces with other targets in the cell[22]. The administration of ADMA in rats causes an
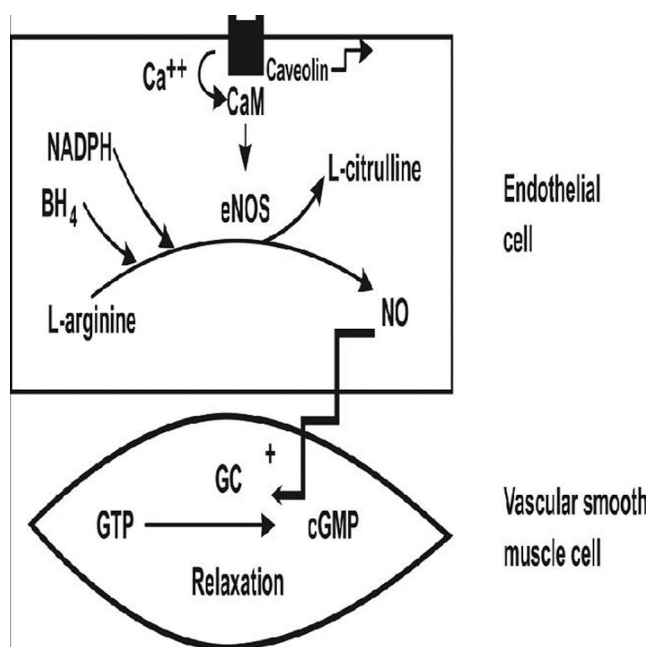

Figure 1 - Production of NO by endothelial cells. Modified from Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation.* 2004;109 (suppl III): III27- III32. BH_4 = tetrahydrobiopterin.

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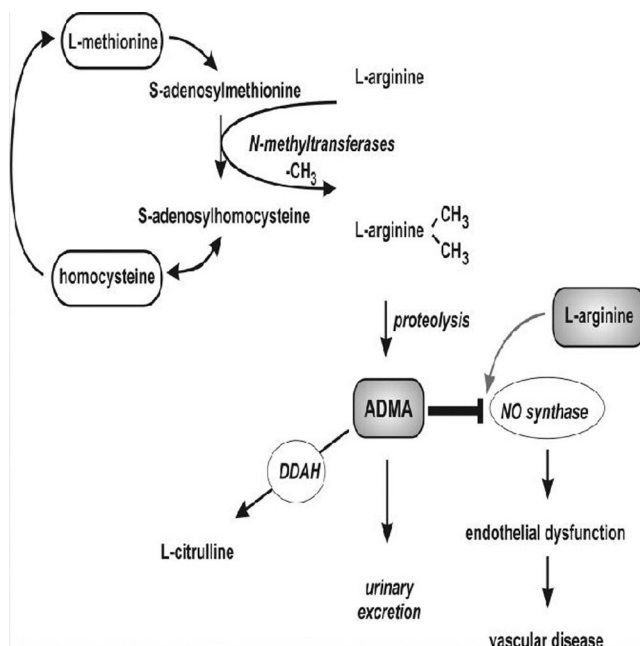Landim MBP et al.

Figure 2 - Schematic view of the biochemical pathways related to ADMA. Modified from Böger RH. Asymmetrical dimethylarginine (ADMA): a novel risk marker in cardiovascular medicine and beyond. Annals of Medicine. 2006;38:126-36.

increase in the renal vascular resistance and blood pressure,[23] confirming its biological action *in vivo*. Its levels are much higher intracellularly than extracellularly, sufficient in some cases to inhibit NOS, as demonstrated with cultivated endothelial cells.[24] However, an independent additional action modality was demonstrated *in vivo*, in which the chronic infusion induced vascular injuries in eNOS knockout mice.[25] Additionally, the three methylarginines interfere with the transport of L-arginine as mediated by the cationic amino acid carrier within the plasma membrane (y+ channels), explaining the SDMA inhibitor effect in the context of NO generation.[26]

Renal excretion is partly responsible for the elimination of methylarginines - SDMA is mainly involved, but ADMA and L-NMMA are also extensively metabolized and produce citrulline and dimethylamine by the action of the dimethylarginine dimethylaminohydrolases (DDAHs).[27] This enzymatic group presents in superior organisms two isoforms codified by genes on chromosomes 1 (DDAH-1) and 6 (DDAH-2), with distinct tissue-relevant distributions but seemingly similar activities.[28]

The regulation of gene expression and DDAH activity remain largely unclear. Incubating endothelial cells with tumor necrosis factor–alpha (TNF-alpha) or oxidized low density lipoprotein (LDL-ox) decreases the activity of this enzyme.[29] Other factors, such as the oxidative stress associated with S-nitrosylation and high levels of glucose and homocysteine, contribute to diminishing DDAH activity, leading to an elevation of ADMA levels.[30,31]

Elevated ADMA in the context of renal insufficiency occurs in a variety of ways, probably because the DDAH activity can vary under different conditions.[32] This molecule is dialyzable, but it reaches pathological concentrations after dialysis process.[33] In the case of chronic renal insufficiency, it fulfills the criteria of an uremic toxin because it increases in inverse proportion to diminishing renal function. It is a guanidine compound, and a product of protein metabolism. Multiple biological functions can be impacted by inhibiting NOS, including the cardiovascular, osseous, and immune systems.[20]

Subsequent research has also suggested the involvement of the liver in the metabolism of dimethylarginines. Researchers have demonstrated that hepatocytes abundantly express y+ channels in their membranes and contain high concentrations of DDAH.[34,35] A more recent study carried out in patients who underwent hepatic surgery confirmed the entrance of ADMA in the liver.[36] These and other metabolic studies have demonstrated that the liver is essential in regulating plasma concentrations of dimethylarginine.

ADMA and cardiovascular risk

Patients with chronic renal insufficiency have an increased risk of atherosclerotic vascular disease, and their ADMA levels are elevated. In those with end-stage renal disease, ADMA has been independently associated with the intima-media thickness of the carotid artery and may predict its progression during a one-year follow-up period.[37] In a cohort of hemodialysis patients, scientists found that plasma ADMA may be a strong and independent risk factor of total mortality and cardiovascular outcome.[38]

The experimental atherosclerosis induced in animals such as rabbits[39] and monkeys[40] on account of a hypercholesterolemic diet associates itself with high plasma levels of ADMA and endothelial dysfunction. In humans, evidence suggests a positive correlation between ADMA and cholesterol levels. In a group of hypercholesterolemic asymptomatic individuals, the plasma concentration of ADMA was almost two-fold higher than in the normocholesterolemic individuals of the same age. In associated groups, ADMA exhibited a positive association with LDL cholesterol;[41] however, other studies have since failed to show this last association.[42] As discussed above, LDL oxidation in the vascular wall may be responsible for the effect of this lipoprotein on ADMA elevation, through inhibition of DDAH activity. Accordingly, ADMA may increase the expression of oxidized LDL lecithin-like receptor–1 of (LOX-1), the main receptor for LDL-ox in endothelial cells.[43]

The increase in homocysteine can raise the risk of atherogenesis by direct inhibition of the activity of DDAH.[31]

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On the other hand, we note the arginine methylation cycle (Figure 2) - in which the S-adenosylmethionine is the methyl donor to the arginine, producing the S-adenosylhomocysteine that is ultimately hydrolyzed to homocysteine. An excess of this amino acid can contribute to cardiovascular risk through an increase in ADMA,[31] which is considered an important link between hyperhomocysteinemia and endothelial dysfunction. Since ADMA contains two methyl groups, its synthesis is followed by the generation of two homocysteine equivalents. Recently, a moderate but highly significant positive association was identified between plasma ADMA and homocysteine in a large population study.[44]

The mechanisms of increasing ADMA levels associated with diabetes or in the context of insulin resistance are not well understood. However, an increase in ADMA concentrations has been identified in animal models of type 1 and 2 diabetes and in patients who suffer from type 2 diabetes or insulin resistance.[45] In diabetic rats, the activity of aortic DDAH was significantly reduced and was found to be negatively associated with plasma ADMA concentrations.[46] The induction of type 2 diabetes in rats by streptozotocin raised plasma ADMA levels, and treatment with insulin resulted in its normalization.[47]

Another study showed that, in the absence of symptoms of coronary or peripheral atherosclerotic disease, age, median arterial blood pressure, and resistance to insulin were core determinants of plasma ADMA concentrations.[48] Another study suggested a positive correlation between resistance to insulin and plasma ADMA readings in healthy and non-diabetic individuals.[49]

However, the reports in the literature regarding ADMA concentrations in diabetic individuals are inconsistent. Poorly-controlled high concentrations were recorded in patients suffering from type 2 diabetes,[50] although there have been reports of diminished levels in similar patient groups.[45] High levels were also reported in gestational diabetes mellitus and type 1 diabetes.[51]

In patients with cardiac insufficiency, ADMA levels are high, and this may diminish both ventricular contraction and cardiac rates.[52] However, it remains unclear whether there exists any causative correlation with cardiac or endothelial functions. One study suggested that resting cardiac output as well as increases in induced-exercise output may be energetically attenuated following the infusion of ADMA. This suggests a possible role similar to that in the physiopathology of cardiac insufficiency and may indicate reduced exercise tolerance.[52]

In reviewing prospective clinical studies, we find strong and convincing evidence of the role of ADMA in the development of cardiovascular disease. In a Finish case-control study, high concentrations of ADMA were associated with an increased risk of acute coronary events among middle-aged and non-smoking men, and especially among those with a history of coronary disease.[53] In another study of patients with stable angina, high plasma concentrations of ADMA predicted adverse cardiovascular events following percutaneous coronary intervention.[54]

The multicentric CARDIAC study aimed to evaluate the relationship between plasma ADMA levels and risk of coronary heart disease.[55] This study showed that manifestations of cardiovascular diseases, when associated with others risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and smoking, were concomitant with high plasma concentrations of ADMA. A concentration of over 1.75 micromoles/liter significantly increased the risk, partially supporting the hypothesis that ADMA may be a new marker for cardiovascular disease. All the aforementioned studies should be evaluated cautiously, since they were carried out with specific groups of patients. There exist few prospective general population studies that have meaningfully established the role of ADMA in the hierarchy of both classical risk factors and novel indicators of cardiovascular disease.

ADMA diminishes during normal pregnancies, but it increases in women with preeclampsia.[56] In women with high levels of ADMA at the beginning of the pregnancy, a clear correlation with endothelial dysfunction has been reported. This data only applies to women who subsequently develop preeclampsia,[57] suggesting that ADMA may be a new risk marker for the precocious detection of this complication of the gestation cycle. The precise mechanism of ADMA elevation in these patients has not been well explained to date, but it is thought that changes in renal function may explain the observed differences between normal pregnancy and preeclampsia groups. Another important observation is the involvement of the liver in ADMA processing; hepatic dysfunction is a key determinant of plasma ADMA changes in pregnant women.

In children with pulmonary hypertension and in experimental models of pulmonary hypertension, ADMA tends to be high and DDAH is often abnormal.[58] In patients with idiopathic arterial pulmonary hypertension, ADMA may be an independent predictor of mortality.[59] Administering L-NMMA produces a quick and sustained elevation in pulmonary arterial pressure.[60]

ADMA has a very narrow range of normal concentrations within the general population. When these concentrations are even only slightly increased, ADMA is associated with high cardiovascular risk. Consequently, it is very important to achieve high measurement accuracy[44]. The original measurements were taken using HPLC (High Performance

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Liquid Chromatography), which is still the most common method.[61] The levels in the plasma of healthy adults vary between 0.3 and 1.0 micromole/liter[62] and in the spinal fluid the corresponding concentrations are between 0.01 and 0.07 micromole/liter.[63] Several methods have been outlined using mass spectrometry coupled with a separation system. Such measures have more favorable lower detection limits.[64] Antibodies against ADMA are commercially available and an ELISA method was recently developed, which can recover ADMA from both plasma and serum samples.[65]

ADMA as a therapeutic option for atherosclerosis

Reversing the effects of increased ADMA or the reduction of ADMA levels may be meaningful goals for the treatment of endothelial dysfunction.

Supplementing a patient's diet with arginine, at least theoretically, may efficiently reverse the endothelial dysfunction caused by high levels of ADMA. In one study, arginine improved endothelial function in patients with hypercholesterolemia and increased the achievable walking distance of patients with peripheral vascular disease.[66] In hypercholesterolemic rabbits, the increase in the correlation between L-arginine and ADMA explained, at least partly, how the formation of NO was accelerated by the exogenous administration of L-arginine.[67] The basal plasma concentration of the L-arginine was almost 25 to 30 times higher than the Michaelis-Menten constant (Km) *in vitro* for eNOS. However, exogenous administration of eNOS was shown to recover endothelial function *in vivo* by dampening the effects of ADMA elevation, a probable explanation for the L-arginine paradox.[68] Definitive studies are still necessary, with large, randomized and prospective trials that may show the therapeutic benefits of that amino acid.

Antagonist drugs of the renin-angiotensin-aldosterone system, such as angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers, and aldosterone antagonists, diminish plasma ADMA levels by mechanisms that are unclear. Possible pathways include improvements in oxidative stress as impacted by angiotensin II, a ROS precursor. Comparing ACE inhibitors both with and without antioxidant properties suggests that the former may be more effective in reducing ADMA concentrations[69]. Blood pressure reduction *per se* may also be relevant.

Statins may offer hope for treating endothelial dysfunction and elevated ADMA levels. However, almost every study to date has failed to support this hypothesis. In animals on a hypercholesterolemic diet, lovastatin failed to reduce either ADMA or SDMA.[70] In rats, administering sinvastatin three days prior to LDL failed to alter ADMA plasma levels.[71]

Nevertheless, reports have suggested certain statin effects in the context of human ADMA metabolism and rat aortic ring control. [72,73] Other studies show that the basal levels of ADMA may be impacted by pravastatin in the context of endothelial function improvements.[74] However, further work is required in this realm. The impact of fibrates on ADMA levels was initially demonstrated in rats and with *in vitro* studies.[75] However, the same effects were not observed when in hypertriglyceridemic individuals[76] for reasons that remain unclear. Niacin also influenced the decline of plasma ADMA levels in patients with low HDL cholesterol. The mechanism may have involved the depletion of S-adenosylmethionine.[77]

Oral antidiabetics, such as metformin and thiazolidinediones, reduce ADMA levels[49] by mechanisms that are still not sufficiently clear. Thiazolidinediones have been shown to influence ADMA release from endothelial cells *in vitro* and ADMA concentrations in rats *in vivo*.[78,79] A clinical study with this group of drugs clearly showed a notable reduction in ADMA levels for both hypertensive and non-diabetic individuals. A few studies have also explored these drugs with diabetic patients and diabetic animal models. Metformin has been shown to reduce ADMA levels in type 2 diabetic patients, though this effect may only have been a consequence of better glycemic control. We note with interest that this drug is structurally similar to ADMA and can be transported by y+ channels.[80]

Among natural compounds with antioxidant properties, vitamin E may prevent elevation of ADMA that is induced by the administration of LDL in rats[81]. In humans, this same vitamin decreased plasma ADMA in patients with chronic renal insufficiency.[82] However, to date we have no convincing evidence that would support the clinical use of this vitamin in human beings.

Estrogens are expected to reduce ADMA levels; however, the available clinical evidence remains inconsistent. A positive effect was confirmed in placebo-controlled clinical studies involving hormone replacement therapy with postmenopausal women.[83] Another very important randomized, placebo-controlled clinical assay carried out in healthy post- menopausal women significantly diminished plasma ADMA levels, although the L-arginine/ADMA ratio did not change. The latter result suggests a neutral effect of hormone replacement therapy on ADMA concentrations and NO production.[84] Most of the evidence in the literature suggests no benefit in terms of cardiovascular morbidity or mortality.

Other classes of useful drugs in the management of cardiovascular disease have been assessed for their effects on plasma ADMA levels. Aspirin offers benefits consistent with its antioxidant properties.[85] Reducing vitamins of plasma

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homocysteine failed to reduce ADMA levels concomitant with their homocysteine-relevant effects.[86] Just one clinical study has suggested ADMA-relevant effects when folic acid is administered to hyperhomocysteinemic individuals.[87] Scientists have reported conflicting results in terms of the impact of polysaturated fatty acids on ADMA concentrations. The use of recombinant human erythropoietin increases the quantity of ADMA and reduces DDAH activity in cultivated endothelial cells.[88] These data suggest relevant deleterious effects on the cardiovascular system - ADMA may be an important mediator of such phenomena. We note a recent study of interest in which Gomes et al. demonstrated the benefit of physical training on ADMA levels in individuals with metabolic syndrome.[89]

CONCLUSION

Recent research has yielded promising new insights into endothelial dysfunction in the pathophysiological context of atherosclerosis. Nitric oxide is now understood to be of great importance in maintaining endothelial integrity. The characterization of ADMA as an antagonist molecule of L-arginine, the main substrate of NOS, was a very important step in understanding early-stage atherosclerotic processes. Additional studies, in the realm of both basic science and clinical testing, will be necessary understand the role of this molecule as a promising marker of future coronary events and a possible therapeutic target in the context of atherosclerotic vascular disease.

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