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Brief Review

Does ADMA Cause Endothelial Dysfunction?

John P. Cooke

Abstract—Asymmetric dimethylarginine (ADMA) is an endogenous and competitive inhibitor of nitric oxide synthase. Plasma levels of this inhibitor are elevated in patients with atherosclerosis and in those with risk factors for atherosclerosis. In these patients, plasma ADMA levels are correlated with the severity of endothelial dysfunction and atherosclerosis. By inhibiting the production of nitric oxide, ADMA may impair blood flow, accelerate atherogenesis, and interfere with angiogenesis. ADMA may be a novel risk factor for vascular disease. **(*Arterioscler Thromb Vasc Biol*. 2000;20:2032-2037.)**

Key Words: arginine ■ nitric oxide ■ atherosclerosis ■ vasodilation

NO and Vascular Homeostasis

Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator known, exerting its effect via stimulation of soluble guanylate cyclase to produce cyclic GMP.[1–3] NO is a critical modulator of blood flow and blood pressure.[4–7] It is released by the endothelium in response to shear stress and plays an important role in flow-mediated vasodilation.[4,5] Endothelial release of NO opposes the vasoconstrictor effects of norepinephrine, endothelin, angiotensin II, and serotonin.[8] Pharmacological inhibition or a genetic deficiency of endothelial NO synthase (NOS) impairs endothelium-dependent vasodilation and increases vascular resistance.[6–9] In patients with coronary artery disease, an impairment of NO activity may contribute to ischemic syndromes.[10,11]

Vascular NO also influences vascular structure. NO suppresses the proliferation of vascular smooth muscle.[12] A chronic deficiency or loss of NO activity may contribute to medial thickening and/or myointimal hyperplasia.[13,14] Conversely, treatment with NO donors or gene therapy with NOS reduces lesion formation after vascular injury in animal models.[15,16] Furthermore, NO inhibits the interaction of circulating blood elements with the vessel wall. Platelet aggregation and leukocyte adherence are unlikely when the endothelium is healthy.[17–19] The loss of NO activity accelerates the development of vascular lesions.[20,21] A loss of NO activity occurs early in the course of human vascular disease[22,23] and is a contributing factor to abnormal vasomotion and ischemic symptoms.[10,11] In addition, there is accumulating evidence that the deficit of NO participates in the initiation and progression of atherosclerosis. Intriguingly, very recent data indicate that defective endothelial vasodilator function is predictive of vascular events.[24] Accordingly, there is a compelling clinical rationale to understand the mechanisms of endothelial dysfunction.

Endothelial Dysfunction Is Multifactorial

The mechanisms of endothelial vasodilator dysfunction are multifactorial and dependent on the nature of the vascular disorder. Because of the multifactorial nature of endothelial dysfunction, therapy targeted at restoring endothelial function must be informed by an understanding of the pathophysiology. Endothelial vasodilator dysfunction may be due to increased vasoconstrictor and/or reduced vasodilator influence. Of the causes of reduced vasodilator influence, derangements of the NOS pathway have been most studied. Derangements of the NOS pathway may be categorized as reductions in (1) NO half-life, (2) sensitivity to NO, (3) NOS expression, or (4) NOS activity. Experimental evidence exists for each of these mechanisms.

Increased vascular elaboration of superoxide anion is an abnormality commonly associated with atherosclerosis and its risk factors.[25] The half-life of NO is reduced under conditions of oxidative stress.[26] The attendant formation of peroxynitrite anion produces lipid peroxidation and nitrosation of tyrosine moieties, thereby disrupting cell membranes, cell signaling, and cell survival.[27] Conversely, antioxidant strategies lengthen NO half-life, increase NOS expression, and restore endothelial vasodilator function.[28–30] The oxidative enzymes responsible for increased oxidative stress in the vessel wall include NAD(P)H oxidase, xanthine oxidase, and NOS itself. Under conditions of reduced availability of L-arginine (the NO precursor) or tetrahydrobiopterin (an NOS cofactor), the preferred substrate of the monomer is oxygen, producing superoxide anion.[31–35] Antioxidants may enhance the activity of NOS by preserving tetrahydrobiopterin.[36]

In the later stages of atherosclerosis, reduced sensitivity to endogenous and exogenous NO is observed, possibly due to oxidative inactivation of NO and/or soluble guanylate cyclase. In addition, in advanced atherosclerosis, reduced ex-

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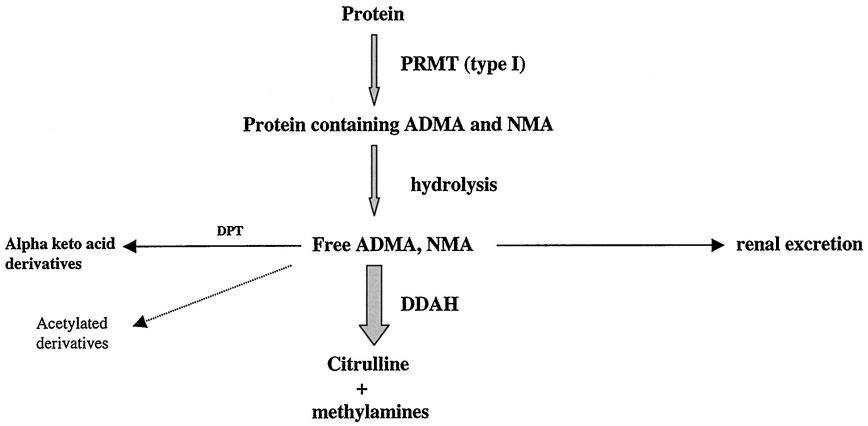

Figure 1. Metabolism of ADMA. Methylated arginines are derived from the breakdown of proteins that have been acted on by enzymes known as PRMTs. PRMT type I methylates proteins that, when hydrolyzed, release ADMA and NMA. PRMT type II methylates proteins that, when hydrolyzed, release SDMA and NMA. DPT is expressed in the kidney and can utilize ADMA, NMA, or SDMA.[92] Acetylated derivatives of methylated arginines have been found in human urine, but the pathways responsible have not been delineated. Light arrows refer to minor metabolic pathways. Heavy arrows refer to major pathways.

pression of the endothelial isoform of the NOS enzyme is observed, possibly due to cytokine- or lipid-induced instability and/or reduced transcription of NOS mRNA.[37,38] Additionally, certain polymorphisms of the NOS gene may be associated with functional alterations in the enzyme and vascular disease.[39] Finally, a growing body of data indicates that endogenous inhibitors of NOS may be responsible for endothelial vasodilator dysfunction in many individuals with coronary and peripheral arterial diseases and in those with their risk factors, particularly hypercholesterolemia, hyperhomocysteinemia, tobacco use, and aging. The endogenous inhibitors are asymmetric dimethylarginine (ADMA) and *N*-monomethylarginine (NMA). Because the former is the predominant species (plasma levels of ADMA are 10-fold greater than those of NMA), further discussion will focus on ADMA.

ADMA Is an Endogenous Inhibitor of NOS

It has long been known to biochemists that methylated arginines are excreted in the urine.[40] However, Vallance et al[41] recognized the physiological significance of this observation and were the first to demonstrate that endogenous ADMA antagonized endothelium-dependent vasodilation. They observed a 9-fold elevation of plasma ADMA in patients with renal failure. Intriguingly, plasma from patients with renal failure induced vasoconstriction of vascular rings in vitro, an effect that was reversed by the addition of L-arginine to the medium. The marked elevation in plasma ADMA may explain the severe endothelial impairment of patients with renal failure. In these patients, dialysis normalizes ADMA levels, an effect that is temporally related to an improvement in endothelium-dependent vasodilation.[42,43] Administration of L-arginine to patients with renal failure also restores endothelial function.[43]

Subsequently, plasma ADMA has been found to be elevated in patients with vascular disease, as well as in the setting of risk factors for vascular disease.[44–49] Hypercholesterolemic animals and humans manifest an impairment of endothelium-dependent vasodilation.[49–51] In these individuals, plasma ADMA levels are better correlated with endothelial dysfunction than are LDL cholesterol levels.[49] Furthermore, the endothelial vasodilator dysfunction associated with an elevated plasma ADMA level is reversible by administration of L-arginine, consistent with the notion that ADMA is a competitive inhibitor.[49]

Intriguingly, plasma levels of ADMA appear to be dynamically regulated and can be correlated with measures of NO synthesis. In the Dahl salt-sensitive rat, a high-salt diet is associated with an increase in urinary ADMA excretion and an increase in blood pressure.[52] In humans with salt-sensitive hypertension, administration of a high-salt diet increases plasma ADMA levels and blood pressure and reduces urinary NO_x; a low-salt diet reverses these abnormalities.[46] Preliminary studies indicate that plasma ADMA also increases with administration of a high-fat diet, which is associated with a temporally related impairment in endothelial vasodilator dysfunction.[53] The mechanisms by which ADMA becomes elevated under these conditions require an understanding of its origin and metabolic fate.

Origin and Fate of ADMA

ADMA is not derived from the methylation of free L-arginine. Rather, ADMA is derived from the catabolism of proteins containing methylated arginine residues (Figure 1). These proteins are largely found in the nucleus and appear to be involved in RNA processing and transcriptional control.[55] There are 2 types of enzymes that methylate arginine residues. These are protein arginine methyltransferase types I and II (PRMT I and PRMT II).[56,57] PRMT type I forms ADMA and NMA, whereas PRMT type II forms symmetric dimethylarginine (SDMA) and NMA. SDMA does not inhibit NOS. There are a number of type I PRMTs, with specificity for different proteins.[55] By contrast, the only known substrate for type II PRMT is myelin basic protein.

When these proteins undergo hydrolysis, their methylated arginine residues are released. Methylated arginines are excreted in the urine.[40] This explains the increase in plasma ADMA levels in patients with renal insufficiency. Methylated arginines may also be metabolized. A minor source of metabolism occurs via dimethylarginine pyruvate transferase in the kidney and possibly, via acetylation in the liver. However, the major metabolic pathway for NMA and ADMA is the enzyme dimethylarginine dimethylaminohydrolase (DDAH).[58] Two isoforms of DDAH are known, I and II. Either or both isoforms have been found in every cell type examined. DDAH I is typically found in tissues expressing

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neuronal NOS, whereas DDAH II predominates in tissues containing the endothelial isoform of NOS.[59]

Dysregulation of DDAH: A Novel Mechanism of Endothelial Dysfunction

DDAH plays an important role in regulating ADMA levels. When SDMA is injected intravenously, 60% is recovered in the urine; by contrast, after intravenous administration, only 5% of ADMA is recovered in the urine.[60] Furthermore, in renal failure, there is a significantly greater increase in plasma SDMA than in ADMA.[61] These observations are explained by the fact that ADMA, but not SDMA, is a substrate for DDAH.[58] ADMA undergoes extensive metabolism in vivo compared with SDMA.

Additional evidence that DDAH is a critical regulator of ADMA levels comes from observations of the effects of the DDAH inhibitor 4124W. Addition of 4124W to an isolated vascular segment induces gradual vasoconstriction, which is reversed by addition of L-arginine to the medium.[62] This finding is most consistent with the view that ADMA is constantly being produced in the course of normal protein turnover. The production of ADMA is balanced by its metabolism by DDAH. Accordingly, inhibition of DDAH activity will cause a gradual accumulation of ADMA, sufficient to induce vasoconstriction.

Recent data from our laboratory indicate that hypercholesterolemia may cause a decline in DDAH activity. The accumulation of ADMA that ensues may contribute to lipid-induced endothelial vasodilator dysfunction. We found that when cultured endothelial cells were exposed to oxidized LDL cholesterol, ADMA accumulated in the medium at a faster rate than when cells were treated with vehicle or native LDL cholesterol.[63] The accelerated accumulation of ADMA was associated with a temporally related decline in DDAH activity. Similarly, the activity of DDAH is reduced in both vascular and nonvascular tissues of hypercholesterolemic rabbits (in which the animals' plasma ADMA levels are known to be elevated).[63] More recently, we have made similar observations in vitro and in vivo regarding the adverse effect of hyperglycemia to reduce DDAH activity and to increase ADMA accumulation (J.P.C. et al, unpublished observations, 2000). Furthermore, the decline in DDAH activity appears to be related to oxidative stress and can be prevented by the use of antioxidants.

Does ADMA Explain the Arginine Paradox?

The "arginine paradox" refers to the discordance between observations made in vitro and those made in vivo regarding the sensitivity of NO synthesis to arginine availability. Studies of a partially purified preparation of NOS in a cell-free system indicated that the K_m of NOS for L-arginine was in the micromolar range.[64] Accordingly, L-arginine should not be rate-limiting for NO synthesis because it is in the 50 micromolar range in plasma and in the millimolar range within the endothelial cell. Indeed, in normal animals and humans, most investigators report no effect of L-arginine on endothelial vasodilator function. Yet under certain circumstances, L-arginine does seem to be rate-limiting. This is most apparent in animal models or in patients with hypercholesterolemia and/or atherosclerosis, wherein endothelium-dependent vasodilation is impaired. There is a high degree of concordance among investigators that under these conditions, administration of L-arginine improves endothelium-dependent vasodilation and increases NO synthesis.[49,65–71] Moreover, L-arginine relieves symptoms and improves exercise tolerance in patients with coronary and peripheral arterial disease. Indeed, the weight of the evidence indicates that there is a nutritional requirement for supplemental L-arginine in these individuals.

The elevation of plasma ADMA provides a possible explanation for the benefits of supplemental L-arginine in these patients. The plasma level of ADMA is normally $\approx$1 μmol/L, is typically increased 2-fold in subjects with risk factors for vascular disease, and is increased even further (up to 10-fold) in patients with clinical atherosclerosis. But with circulating L-arginine levels at $\approx$50 μmol/L, is this elevation in ADMA sufficient to have an effect on NOS? Perhaps the sensitivity to ADMA could be explained by the fact that it also competes with L-arginine for uptake by the y+ transporter. Furthermore, the y+ transporter and endothelial NOS are physically associated in the caveolae of endothelial cells.[72] The arginine interventions discussed above certainly support the view that NO synthesis is sensitive to changes in extracellular arginine availability. Furthermore, Faraci and colleagues[73] have shown that the K_m of NOS isolated from the cerebellum (largely neuronal NOS) is 2.8 μmol/L. Also, concentrations of 1 to 10 μmol/L ADMA were sufficient to cause modest contractions of cerebral vessels in situ.

Intravenous infusion of ADMA sufficient to increase plasma concentrations 9-fold increased systolic blood pressure by 15% in anesthetized guinea pigs.[41] Intra-arterial infusion of ADMA (8 μmol/min) reduced forearm blood flow by $\approx$30% in healthy volunteers.[41] Finally, we have observed intriguing changes in endothelial behavior when cultured cells are chronically exposed to concentrations of ADMA and L-arginine similar to those found in the plasma of hypercholesterolemic individuals. ADMA-exposed cells increase the adhesiveness of monocytes in coculture.[74] Furthermore, monocytes and T lymphocytes derived from hypercholesterolemic individuals are hyperadhesive, an abnormality that is reversed by several weeks of oral administration of L-arginine.[74] This finding is consistent with previous observations in hypercholesterolemic animal models and humans that administration of the NO precursor inhibits endothelial-monocyte interaction.[18,75,76] These observations raise the following question.

Is ADMA a Risk Factor for Vascular Disease?

Preclinical studies suggest that NO is a potent, endogenous, antiatherogenic molecule, suppressing key processes in atherosclerosis. This view is supported by the fact that pharmacological inhibition or a genetic deficiency of endothelial NOS accelerates atherogenesis in animal models.[20,21,77] Does *endogenous* ADMA accelerate atherosclerosis? Our earlier observations that chronic administration of L-arginine can slow and even reverse the progression of vascular lesions are consistent with this hypothesis.[78–86] The hypothesis is further supported by the observation that supplemental dietary argi-

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nine enhances NO synthesis in the rabbit aorta, as measured directly by chemiluminescence.[18]

Intriguingly, in animal models and in humans, endogenous ADMA levels may be predictive of vascular lesion formation. After balloon injury, the regenerating endothelial cells manifest higher intracellular levels of ADMA and impaired endothelium-dependent vasodilation.[87–89] The severity of the endothelial dysfunction and the intracellular levels of ADMA are directly related to the intimal thickness of the injured vessel.[87,89] There are similar data for humans. In 120 Japanese individuals with varying levels of risk, intimal-medial thickness of the carotid artery was measured by ultrasound and was correlated with blood pressure, lipid profile, smoking history, blood sugar, age, and ADMA. A multivariate analysis revealed that ADMA and age were the only independent predictors in these patients.[90]

Finally, 3 groups have independently offered evidence that endothelial vasodilator dysfunction is an independent predictor of vascular events.[24,91,92] Impaired coronary blood flow response to acetylcholine or reduced brachial artery response to flow was predictive of vascular morbidity and mortality. To the extent that ADMA is responsible for the impairment of endothelial vasodilator dysfunction in these patients, it may be a predictor for vascular events.

Future Directions

Work in this area would be facilitated by an improved assay. Currently, detection of ADMA is labor-intensive and requires its derivatization to a fluorescent probe, followed by high-pressure liquid chromatography combined with a fluorescent detector. High-throughput and reproducible assays are needed and may arise as immunoassays or enzymatic assays. These assays will facilitate determination of the clinical significance of ADMA as a contributor to pathophysiology and symptoms and as a risk factor for vascular events.

The regulation of DDAH is just beginning to be understood, and modulators of its expression are being defined. Structure-function studies and novel drug discovery will be enhanced by obtaining its crystal structure. Genetically engineered animals that overexpress or are deficient in DDAH are being created and will provide new insights into the developmental and physiological actions of DDAH. Other enzymes along the metabolic pathway of ADMA deserve further scrutiny; for example, can increased methylation of specific proteins or increased catabolism of these proteins be involved in elevation of plasma ADMA?

Other interesting biological questions remain to be answered. Does ADMA play an important regulatory role in inflammation or infection (ie, as a “brake” on the action of inducible NOS)? Does ADMA have a role in the central or peripheral nervous system? Are other processes that are modulated by NO (eg, angiogenesis) affected by endogenous ADMA? There are many unanswered questions, but it seems certain that endogenous NOS inhibitors represent an important new class of biological mediators. An understanding of their physiological and pathological roles and their regulation may lead to new therapeutic avenues.

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