Asymmetrical Dimethylarginine
The Über Marker?

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The traditional risk factors of hypercholesterolemia, hypertension, diabetes mellitus, and tobacco exposure identify a subset of patients at greater cardiovascular risk. A variety of clinical phenotypes, biochemical markers, and genetic polymorphisms have been proposed to explain the variance in risk not explained by the traditional factors. Notably, all of the traditional risk factors, as well as the great majority of new risk markers, are associated with endothelial vasodilator dysfunction.

Because the end points (endothelial dysfunction leading to plaque formation, progression, and rupture) are the same, it follows that diverse risk factors ultimately share common pathways(s) of pathobiology. We and others have provided evidence for a ubiquitous mechanism of endothelial pathobiology shared by all risk factors and markers examined to date. This mechanism of endothelial derangement is mediated by an endogenous inhibitor of nitric oxide synthase (NOS), a molecule known as asymmetrical dimethylarginine (ADMA). Risk factors impair endothelial vasodilator function by causing the accumulation of ADMA. Furthermore, by blocking NO generation, ADMA initiates and promotes processes involved in atherogenesis, plaque progression, and plaque rupture. This review examines the burgeoning body of literature that supports ADMA as an “Über marker,” a biochemical factor mediating the adverse vascular effects of many other risk factors and markers.

ADMA: A Major Cause of Endothelial Dysfunction

Endothelial NOS converts the amino acid L-arginine into L-citrulline and NO. The importance of NO in vascular homeostasis has been discussed elsewhere. In addition to its vasodilator activity, NO inhibits key processes involved in vascular disease, including leukocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. In animal models, alterations in vascular NO synthesis profoundly influence the progression of atherosclerosis and restenosis. These experimental observations have gained greater significance with recent reports that impairment of the NOS pathway independently predicts cardiovascular events.

Major causes of impairment of the NOS pathway are the endogenous NOS inhibitors ADMA and N-monomethylarginine (MMA). Plasma levels of ADMA are 10-fold greater than those of MMA. Because it is the predominant species in plasma, most studies to date have focused on ADMA. The importance of ADMA as an endogenous inhibitor of NOS was first recognized by Vallance and colleagues in patients with end-stage renal disease. In these patients, ADMA accumulates as a result of reduced renal clearance. Dialysis reduces plasma ADMA levels and normalizes endothelial function. Associations between increased levels of ADMA and many cardiovascular risk factors such as age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, and hyperhomocystinemia have been documented. Furthermore, evidence for a causal relationship between increased ADMA levels and endothelial vasodilator dysfunction has been demonstrated in many of these conditions. In hypercholesterolemic adults (but not children), elevated ADMA levels are inversely correlated with endothelium-dependent vasodilation in the forearm. Consistent with the notion that ADMA is a competitive inhibitor, in hypercholesterolemic adults an intravenous infusion of L-arginine restores endothelial function and increases urinary nitrate excretion (a surrogate parameter of NO production).

Plasma ADMA levels can change quite rapidly in humans, temporally associated with alterations in endothelial vasodilator function. A single high-fat meal doubled ADMA levels in diabetic patients and was temporally associated with a significant impairment of flow-mediated endothelium-dependent vasodilation in the forearm. A single oral dose of methionine increases plasma homocysteine levels, paralleled by an increase in plasma ADMA and a decline in endothelium-dependent vasodilation. In humans with salt-sensitive hypertension, administration of a high-salt diet increases plasma ADMA and blood pressure and reduces urinary nitrogen oxides. A low-salt diet reverses these abnormalities.

ADMA Regulates Vascular Resistance

There is substantial evidence that ADMA regulates vascular resistance in humans. Intra-arterial infusion of ADMA reduces endothelium-dependent vasodilation in the human forearm. Moreover, intravenous infusion of ADMA (to increase plasma ADMA levels approximately 3-fold) increases sys-
temic vascular resistance by approximately 24% in healthy
tissue.23 Elevated plasma levels of ADMA are
dependent of the increased renovascular resistance observed
with aging, hypertension, and heart failure.24,25 In patients
with renal failure, plasma ADMA levels correlate directly
with left ventricular thickness and inversely with ejection
fraction,26 which is consistent with the effect of ADMA to
increase systemic resistance.

In normal pregnancy, there is an initial fall in blood
pressure and subsequent return toward baseline values in the
third trimester. These changes in blood pressure are mirrored
by similar changes in plasma ADMA values. In women who
develop preeclampsia, plasma ADMA values are elevated.27
Intriguingly, the impairment of maternal endothelial function
and the elevation of plasma ADMA occur before clinical
evidence of preeclampsia.28

These clinical studies are supported by experimental studies
with cell culture or isolated vessels demonstrating that
ADMA inhibits endothelium-dependent vasodilation and/or
NO synthesis.1,29 In mice genetically engineered to express
low plasma ADMA levels, NO synthesis is increased, and
vascular resistance is reduced.30

ADMA and Vascular Structure

By reducing the activity of endothelial NOS, ADMA may
affect vascular structure as well as vascular reactivity. Endo-
thelial cells resurfacing an injured vessel manifest higher
intracellular levels of ADMA and impaired endothelium-
dependent vasodilation.31 The severity of the endothelial
dysfunction and the intracellular levels of ADMA are directly
related to intimal thickness of the injured vessel.5,31 A recent
clinical trial is consistent with a role of ADMA in restenosis.
Patients undergoing coronary angioplasty and stent place-
ment received a single intramural delivery of L-arginine or
vehicle. Intravascular ultrasound at 6 months revealed a 36%
reduction of neointimal volume in those patients receiving
L-arginine.32

Exposure of endothelial cells in culture to pathophysiolog-
ically relevant concentrations of ADMA reduces NO synthe-
sis, increases superoxide generation, and increases the adhe-
siveness of endothelial cells for monocytes.29 Evidence also
exists in humans that ADMA enhances endothelial-monocyte
interaction. Mononuclear cells of hypercholesterolemic indi-

ciduates are hyperadhesive, an abnormality that is positively
correlated with carotid intima-media thickness and were
predictive for progression of disease.38 Intimal thickening in
uterine arteries after hysterectomy is correlated to plasma
ADMA levels.39

As expected of a factor that may adversely affect vascular
structure, plasma ADMA levels are associated with cardio-
vascular complications such as stroke, congestive heart fail-
ure, or peripheral arterial disease.40,42 In peripheral arterial
disease, plasma ADMA levels are related to the severity of
disease.42 Notably, an intravenous infusion of L-arginine
significantly improves limb blood flow and pain-free walking
distance in patients with peripheral arterial disease.43 Cere-
brovascular disease is the second most common cause of
dementia after Alzheimer disease, which also may have a
vascular component. In this context, plasma ADMA levels
are reportedly elevated in patients with dementia, associated
with a reduction in plasma nitrogen oxides.44

Plasma ADMA levels may be predictive of cardiovascular
events and/or mortality. In critically ill patients on a surgical
intensive care unit, elevated plasma ADMA values were
associated with an adverse outcome.45 In nonsmoking men
with a history of coronary heart disease, those in the upper
quartile of ADMA levels had a 4-fold increased risk of an
acute coronary event.11 In patients with end-stage renal
disease, ADMA levels emerged as the second strongest
predictor of all-cause mortality after age, outweighing estab-
lished risk factors such as hypertension, diabetes, hypercho-
lesterolemia, or smoking.46 These small studies suggest that
plasma ADMA may be an independent risk factor for
vascular disease. However, its value as a prognostic indicator
needs to be validated in large, prospective clinical trials that
are now under way.47

Mechanisms by Which ADMA
Becomes Elevated

Generation of ADMA

ADMA is not derived from the methylation of free
L-arginine. Rather, ADMA is generated from posttransla-
tional modification of arginine residues within a variety of
specific proteins that are predominantly found in the cell
nucleus (for review, see Tran et al48). Methylation of
arginine residues is catalyzed by a group of enzymes
termed protein arginine N-methyltransferases (PRMTs)
(Figure). When the proteins undergo proteolysis, free
methylarginines are released.

Two distinct PRMT activities (PRMT types I and II) have
been classified. Both subtypes can monomethylate arginine to
form MMA but differ in that type I asymmetrically dimethy-
lates arginine to form ADMA, whereas type II catalyzes a
symmetrical dimethylation of arginine to form SDMA.48
Whereas ADMA and MMA each inhibit NOS, SDMA is not
capable of doing so.

To date, there is scant evidence that elevated plasma levels
of ADMA are due to increased methylation of arginine
residues. The methylation of arginine residues on proteins is
a highly regulated process, and methylated proteins have a
wide range of functions. Although PRMT activity is influ-
enced by oxidized lipoprotein in vitro,49 it is unlikely that
The first evidence that DDAH is a critical regulator of the NOS pathway came from observations regarding the DDAH inhibitor, 4124W. Addition of 4124W to an isolated vascular segment induces a gradual vasoconstriction, which is reversed by addition of l-arginine to the medium.55 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, pharmacological inhibition of DDAH activity causes ADMA to accumulate, to disrupt NO synthesis, and to thereby induce vasoconstriction.

We have shown that impaired DDAH activity is a central mechanism by which cardiovascular risk factors disrupt the NOS pathway. The activity of DDAH is impaired by oxidative stress, permitting ADMA to accumulate. A wide range of pathological stimuli induces endothelial oxidative stress such as oxidized LDL cholesterol, inflammatory cytokines, hyperhomocysteinemia, hyperglycemia, and infectious agents. Each of these insults attenuates DDAH activity in vitro and in vivo.54–58 The attenuation of DDAH allows ADMA to accumulate and to block NO synthesis (Figure). The adverse effect of these stimuli can be reversed in vitro by antioxidants, which preserve the activity of DDAH.

The sensitivity of DDAH to oxidative stress is conferred by a critical sulfhydryl in the active site of the enzyme that is required for the metabolism of ADMA. This sulfhydryl can also be reversibly inhibited by NO in an elegant form of negative feedback.59 We have shown that homocysteine mounts an oxidative attack on DDAH to form a mixed disulfide, inactivating the enzyme.60 By oxidizing a sulfhydryl moiety critical for DDAH activity, homocysteine and other risk factors cause ADMA to accumulate and to suppress NO activity.

In apolipoprotein E–deficient mice, hypercholesterolemia is associated with increased levels of plasma ADMA and attenuated angiogenesis.60 The effect of ADMA on angiogenesis can be reversed by administration of supplemental l-arginine. These data are consistent with previous observations disclosing a critical role of endothelium-derived NO in angiogenesis.61 The role of ADMA in modulating angiogenesis was strengthened by the finding that C6 glioma cells genetically engineered to constitutively overexpress the enzyme DDAH resulted in tumors that were more vascular and grew faster than wild type.62 Expression of DDAH can also be increased by exposing endothelial cells to retinoic acid. This effect is associated with reduced accumulation of ADMA and increased endothelial cGMP levels.63

In experimental models of pulmonary hypertension, a reduction in pulmonary DDAH activity or expression is associated with an increase in plasma ADMA levels and reduced pulmonary NO synthesis.64,65 A reduction in DDAH activity could explain elevated plasma ADMA levels and l-arginine responsiveness in patients with pulmonary hypertension.66–68

The critical role of DDAH activity in regulating NO synthesis in vivo was convincingly demonstrated by our group with the use of a transgenic DDAH mouse.60 In this animal, the activity of DDAH is increased, and plasma ADMA levels are reduced by 50%. The reduction in plasma ADMA is associated with a significant increase in NOS activity, as plasma and urinary nitrate levels are increased 2-fold. The increase in NOS activity translates into a 15-mm Hg reduction in systolic blood pressure in the transgenic mouse. This study provides compelling evidence for the importance of DDAH activity and plasma ADMA levels in the regulation of NO synthesis.

Biochemical pathway for generation, elimination, and degradation of ADMA. ADMA derives from methylation of arginine residues in proteins. The reaction is catalyzed by PRMTs that transfer a methyl group from S-adenosyl-L-methionine (SAM) to each guanidino nitrogen of an arginine residue. This reaction results in a methylated arginine derivative (protein containing ADMA) and S-adenosyl-L-homocysteine (SAH). Hydrolysis of the methylated proteins releases ADMA. ADMA is a competitive inhibitor of endothelial NOS. All methylarginines are excreted into the urine.50 This may be due to persistent impairment in the activity of dimethylarginine pyruvate aminotransferase (DPT). The major metabolism of ADMA occurs via degradation through the enzyme DDAH. The enzyme DDAH hydrolyzes ADMA to form dimethylamine and L-citrulline. DM indicates diabetes mellitus; HTN, hypertension, LDL-C, LDL cholesterol; HCY, hyperhomocysteinemia; and CMV, cytomegalovirus.
ADMA and the “l-Arginine Paradox”

The l-arginine paradox relates to observations first made by our group that the administration of l-arginine can reverse endothelial vasodilator dysfunction under some conditions. Later, biochemists who had purified NOS enzyme found that its $K_m$ for l-arginine was in the range of approximately 5 $\mu$mol/L. Because l-arginine plasma levels are in the range of 50 $\mu$mol/L, it was paradoxical that l-arginine could be rate limiting. Possible explanations for this phenomenon include nonenzymatic generation of NO from l-arginine, release of growth hormone or insulin, or effects at the level of the y’-transporter responsible for cellular uptake of l-arginine. However, reversal of the effect of ADMA represents a more likely mechanism.

Endothelial cells exposed for 24 hours to concentrations of ADMA that exist in the plasma of hypercholesterolemic individuals generate less NO and more superoxide anion and are more adhesive for monocytes. Similarly, a reduction of plasma ADMA levels from 1.6 $\mu$mol/L (in normal mice) to 0.7 $\mu$mol/L in our transgenic DDAH mice is associated with a 2-fold increase in plasma and urinary nitrogen oxides. The striking effect of modest changes in plasma ADMA is surprising given the high intracellular concentrations of l-arginine, which are almost 3 orders of magnitude greater. However, intracellular l-arginine may not have access to NOS (intracellular compartmentalization). Indeed, eNOS colocalizes with plasma membrane regions referred to as caveolae.

Finally, there is evidence that ADMA may also “uncouple” endothelial NOS, such that molecular oxygen becomes the substrate for electron transfer rather than arginine. Under these conditions, endothelial NOS generates superoxide anion to increase oxidative stress, attenuate NO bioactivity, and induce additional endothelial dysfunctions. However, an elevation of plasma ADMA does not necessarily predict a beneficial response to l-arginine supplementation. In a small study of men with stable angina and elevated plasma ADMA levels, oral l-arginine supplementation did not improve forearm endothelial vasodilator function, treadmill walking time, or time to ST segment depression. The lack of benefit of l-arginine in this study likely reflects the multifactorial mechanisms of endothelial vasodilator dysfunction. For example, NOS may become a major source of superoxide anion and endothelial dysfunction when l-arginine availability becomes rate limiting. However, deficiencies or reduced activity of tetrahydrobiopterin, heat shock protein 90, or specific tyrosine kinases would probably not be addressed by supplementation with l-arginine. L-Arginine may not be useful in later stages of atherosclerosis, in which cytokine- or lipid-induced instability and/or reduced transcription of NOS may decrease its expression. Endothelial dysfunction secondary to certain NOS gene polymorphisms might be unresponsive to supplemental l-arginine.

Therapeutic Modulation of Plasma ADMA

Notably, plasma ADMA can be reduced by pharmacotherapy. When glycemic control is improved by administration of metformin, plasma ADMA levels are reduced in diabetics. In patients with insulin resistance, rosiglitazone improved insulin resistance and lowered plasma ADMA levels. Treatment of hypertensive patients with a converting-enzyme inhibitor or angiotensin receptor antagonist reduces plasma ADMA levels. Estrogen therapy, alone or with progesterone, modestly reduces plasma ADMA (but not SDMA) levels, an effect that may be due to estrogen-induced increases in DDAH activity. However, hormonal replacement may also reduce plasma l-arginine levels, so that the l-arginine/ADMA ratio is not favorably affected. Strategies to reduce oxidative stress would likely enhance DDAH activity and thereby reduce plasma ADMA levels, but the results of 2 small studies of antioxidant vitamins have been negative or inconclusive.

In the future, a likely target for pharmacotherapy is the enzyme DDAH.

Conclusion

To summarize, there is compelling evidence that ADMA plays an important role in the regulation of vascular tone by acting as an endogenous inhibitor of NO synthesis. By inhibiting NO synthesis, plasma ADMA may reduce vascular compliance, increase vascular resistance, and limit blood flow. Furthermore, plasma ADMA may promote atherogenesis as it opposes the vasoprotective effects of NO. Thus, elevations in plasma ADMA may accelerate the progression of atherosclerosis and increase the risk of cardiovascular events. ADMA may mediate the effect of many risk factors and risk markers on the NOS pathway. Plasma ADMA level may be an “Über marker,” reflecting the summative effect of various risk factors on endothelial health.

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References


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