

Asymmetric dimethylarginine: A cardiovascular risk factor in renal disease?

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Asymmetric dimethylarginine: A cardiovascular risk factor in renal disease? Endothelial dysfunction due to reduced availability of nitric oxide (NO) is an early step in the course of atherosclerotic vascular disease. NO is synthesized from the amino acid L-arginine by the action of the NO synthase (NOS), which can be blocked by endogenous inhibitors such as asymmetric dimethylarginine (ADMA). In laboratory animals, administration of ADMA significantly reduces NO generation, and causes an increase of blood pressure and renal vascular resistance. In clinical studies, a strong correlation between increased ADMA blood levels and impaired endothelial-dependent vasodilatation, and cardiovascular morbidity and mortality has been documented in different populations, including in patients with renal disease. Thus, ADMA seems to be the culprit, and not just an innocent biochemical bystander, of the atherosclerotic disease process. Moreover, reduced NO availability is involved in the progression of renal disease, and increased ADMA blood levels may contribute to this process. Interventions that lower ADMA blood levels in renal patients could, therefore, modulate their atherogenic profile and interfere with progression of renal failure.

Nitric oxide (NO) is a very active, but short living, molecule that is released in the circulation from endothelial cells. It is a potent vasodilator that regulates vascular resistance and tissue blood flow. In addition, NO inhibits key processes of atherosclerosis, such as monocyte endothelial adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. Hence, endothelial dysfunction due to reduced NO availability is an early step in the course of atherosclerotic vascular disease [1]. NO is synthesized by stereospecific oxidation of the terminal guanidino nitrogen of the amino acid, L-arginine, by the action of a family of NOS. The synthesis of NO can be blocked, however, by inhibition of the NOS active site with guanidino-substituted analogs of L-arginine, such as ADMA [1].

ADMA is released after posttranslational methylation

from nuclear proteins involved in RNA processing and transcriptional control. The enzyme protein arginine methyltransferase type I (PRMT I) forms ADMA, whereas PRMT II forms symmetric dimethylarginine (SDMA) (i.e., the stereoisomer of ADMA with no known effect on NOS). A number of cell types, including human endothelial cells, elaborate ADMA. The substance is excreted by renal route to some extent, but the major metabolic pathway is degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA to dimethylamine and L-citrulline (Fig. 1). Thus far, two isoforms of DDAH have been characterized; DDAH II is the predominant form in tissues expressing endothelial NOS. Colocalization of DDAH and NOS supports the hypothesis that intracellular ADMA concentration is actively and cell-specifically regulated in NO-generating cells [2]. Since NO has tremendous biological activity, its blood and/or tissue concentration must be kept within narrow limits in order to prevent harmful effects on cell activity. This could be accomplished by cell-specific endogenous NOS inhibitors, such as ADMA, and thus explain the L-arginine paradox. The NO amino acid precursor L-arginine is found in the mammalian organism at concentrations by far exceeding the K_M value for the rate limiting enzyme NOS, so that, theoretically, administration of L-arginine should not enhance NO formation. Several in vivo studies have documented, however, that administration of L-arginine enhances NO production, possibly because the additional L-arginine antagonizes the action of endogenous NOS inhibitors [3].

IS NOS INHIBITION BY ADMA BIOLOGICALLY RELEVANT?

Data from experimental studies document that pathophysiologically relevant ADMA blood levels (between 2–10 $\mu\text{mol/L}$) significantly inhibit NOS in cultured endothelial cells and in isolated human blood vessels, thereby reducing NO generation [1]. Administration of ADMA to

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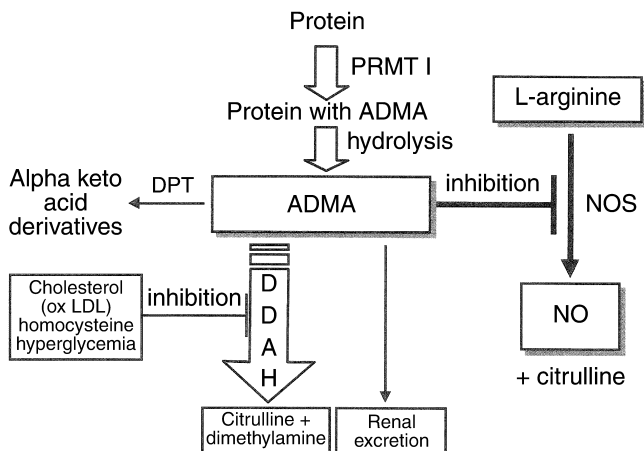


Fig. 1. Biochemical pathway for generation and degradation of ADMA.

laboratory animals caused an increase of blood pressure and renal, mesenteric, and hindquarter vascular resistance [4]. In addition, a recent experiment with transgenic animals harboring the human DDAH gene revealed that increased DDAH activity lowers ADMA blood concentrations and, in parallel, significantly reduces blood pressure (abstract; Dayoub H et al, *Eur Heart J* :132A, 2002). In humans, indirect evidence for a biologically relevant action of ADMA comes from a recent study in renal patients, in whom plasma ADMA levels were increased four times as compared with healthy controls—their blood markedly inhibited NO production in cultured endothelial cells *ex vivo* [5]. Furthermore, Chan et al have found that mononuclear cell adhesiveness significantly correlates with elevated plasma ADMA concentrations in hypercholesterolemic subjects [6]. In cell culture, the adhesiveness of these cells was enhanced by coculturing them with ADMA-stimulated vascular endothelial cells. We were able to demonstrate that systemic administration of ADMA in healthy subjects causes a sustained reduction of NO production and renal perfusion, and increase in blood pressure (abstract; Kielstein JT et al, *Kidney Blood Press Res* 25:130A, 2002). Thus, ADMA is a biologically active NOS inhibitor.

ADMA: A NEW EMERGING CARDIOVASCULAR RISK FACTOR?

A growing number of published clinical studies in non-renal patients documented a strong correlation between increased ADMA blood levels and cardiovascular morbidity and mortality in different populations [1, 7–9]. Results from a cross-sectional study in 116 otherwise healthy subjects revealed that ADMA blood levels were positively correlated with age, mean arterial blood pressure, and glucose intolerance [8]. Most strikingly, plasma

ADMA levels in these subjects were significantly correlated with the intima-media thickness of the carotid arteries as measured by high-resolution sonography. Thus, chronically elevated plasma levels may be of relevance in human vascular pathology (i.e., ADMA being the culprit and not just an innocent biochemical bystander of the atherosclerotic disease process). The cause for the increase of ADMA blood levels in nonrenal populations is not completely understood; however, inhibition of DDAH activity by hypercholesterolemia (especially LDL-cholesterol), hyperglycemia, and hyperhomocystinemia may play an important role [1, 10, 11] (Fig. 1).

ADMA, ATHEROSCLEROSIS, AND PROGRESSION IN PATIENTS WITH RENAL DISEASE

Impaired NO-mediated endothelial-dependent vasodilatation (i.e., the hallmark of early atherosclerosis) has been reported in children and adults with chronic renal failure, and even in patients with adult polycystic kidney disease and normal renal function [12]. Furthermore, L-arginine, but not D-arginine, restores endothelial function in patients with end-stage renal disease (ESRD) independently of hemodialysis treatment [13]. This latter observation points to reduced availability of NO in patients with ESRD.

In 1992, Vallance et al reported increased plasma concentrations of methylarginines (i.e., ADMA and SDMA) in a small group of patients with ESRD [14]. They hypothesized that the high incidence of hypertension and atherosclerosis in patients with terminal renal failure might be caused, at least in part, by dysfunction of the L-arginine/NO pathway secondary to accumulation of ADMA due to declining renal excretion. Indeed, the same authors demonstrated that infusion of ADMA into the brachial artery attenuated endothelial-dependent vasodilatation in healthy volunteers [14]. Several recent studies have confirmed markedly increased plasma ADMA levels in patients with renal failure [15, 16] (Table 1). However, the assumption that decreased urinary excretion is the main cause of elevated plasma ADMA concentrations in ESRD patients has been questioned by the observation that SDMA, which is not degraded by DDAH, accumulates eight times more than ADMA in patients with ESRD. Furthermore, markedly increased plasma ADMA concentrations are found even in patients with incipient renal disease (i.e., patients with normal renal function as documented by measurement of true glomerular filtration rate using inulin clearance) [17]. Interestingly, ADMA levels were elevated independent of the type of renal disease. This observation favors the hypothesis that impaired ADMA degradation by (renal) DDAH, rather than reduced renal excretion, is the main cause of increased plasma ADMA concentrations in patients with renal disease. DDAH is present in abundance

Table 1. ADMA blood levels in renal patients and other populations

Reference	Population	ADMA blood level $\mu\text{mol/L}$
17	Incipient renal disease	4.2 ± 0.9
	Controls	1.4 ± 0.7
14	Chronic renal failure	8.7 ± 0.7^a
	Controls	1.2 ± 0.1^a
15	End-stage renal disease	6.0 ± 0.5
	Controls	1.0 ± 0.1
6	Hypercholesterolemia	2.1 ± 0.2
	Controls	1.3 ± 0.2
7	Peripheral occlusive disease IV	3.5 ± 0.3
	Controls	1.3 ± 0.1
	Elderly subjects	3.5 ± 0.1
	Young controls	1.3 ± 0.1

^aDimethylarginines

in endothelial cells within the glomerulus and renal vessels, and particularly in renal tubular cells [2]. It regulates intracellular methylarginine levels, thereby governing cell-specific L-arginine uptake and NO generation in tubular cells. Destruction of DDAH-rich renal tissue could, therefore, theoretically impair renal degradation of ADMA. Whatever the cause of increased plasma ADMA concentrations in patients with renal disease may be, results of recent in vitro studies suggest that ADMA concentrations in patients with ESRD are high enough to sufficiently decrease NO production [5]. Indirect evidence for a pathophysiologic role of ADMA in this population include observations that, in ESRD patients with manifest atherosclerosis, plasma ADMA levels were significantly higher than in renal patients without signs of atherosclerotic disease [15]. Last, but not least, in a prospective study in 225 patients with ESRD, plasma ADMA concentrations were not only significantly related to the severity of carotid atherosclerosis but, in addition, were the second strongest predictor (after age) of cardiovascular mortality among several (traditional) risk factors assessed [16].

Another important aspect of NO inhibition in renal patients is progression of renal disease. Past and more recent experimental studies have revealed that reduced bioavailability of NO plays a critical role in the progression of renal disease, and increased ADMA blood levels may contribute to this process [18]. In this respect, it is of interest that increased plasma ADMA levels predict the increase of blood pressure in elderly subjects, and, even more importantly, they are significantly associated with the age-related decrease in renal perfusion [19]. Further studies are, therefore, warranted to clarify the role of ADMA in the progression of renal disease.

PERSPECTIVE

Since ADMA is thought to be a competitive NOS inhibitor, the logical therapeutic intervention would be

Table 2. Clearance of creatinine, urea, ADMA, and SDMA in patients on maintenance hemodialysis

	Creatinine	Urea	ADMA	SDMA
Clearance mL/min	163 ± 5	175 ± 6	79 ± 8	135 ± 6

Abbreviations are: ADMA, asymmetric dimethylarginine and SDMA, symmetric dimethylarginine.

administration of L-arginine in order to overcome NOS inhibition [1]. To date, a large body of evidence has been accumulated regarding the beneficial role of L-arginine administration on several aspects of endothelial dysfunction, but surprisingly few studies have specifically addressed the potential role of ADMA. Böger et al showed that administration of L-arginine to patients with peripheral vascular disease not only increased L-arginine/ADMA ratio, and thereby NO production, but also ameliorated clinical symptoms in patients [20]. Moreover, recent studies in patients with essential hypertension and type 2 diabetes mellitus have revealed that pharmacologic treatment with ACE inhibitors, AT₁-receptor blockers, and insulin-sensitizing agents may reduce ADMA blood levels, possibly via enhancement of DDAH activity [10].

The results of studies examining the effect of dialysis treatment on ADMA blood levels in patients with ESRD are controversial. Some authors have found a significant reduction of ADMA blood levels after dialysis treatment, while others have not. We have recently explored this issue using a single-pass batch dialysis system that offers a complete collection of the spent dialysate. Despite its relatively low molecular weight, comparable with that of urea, the dialysance of ADMA was considerably lower than that of urea (Table 2). Further studies are warranted in order to evaluate potential mechanisms of ADMA elimination in patients with ESRD.

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