Kidney International, Vol. 59, Suppl. 78 (2001), pp. S-9–S-13

Relationship of asymmetric dimethylarginine to dialysis treatment and atherosclerotic disease

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Relationship of asymmetric dimethylarginine to dialysis treatment and atherosclerotic disease. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide (NO) synthase. Its concentration is elevated in patients with end-stage renal disease (ESRD), in part because it is excreted via the kidneys. In addition, ADMA is degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA to L-citrulline and dimethylamine. Activity of DDAH is decreased by oxidized low density lipoprotein (LDL) or tumor necrosis factor-α (TNF-α) in vitro yielding increased levels of ADMA. Furthermore, plasma levels of ADMA are elevated in hyperhomocyst(e)inemia and in hypertensive patients on a high salt diet. Data from several experimental studies suggest that ADMA concentrations in a pathophysiologically high range (3 to 10 μmol/L) significantly inhibit vascular NO formation by NO synthase in the presence of L-arginine in isolated human blood vessels, cultured macrophages, and in cultured endothelial cells. It has been well demonstrated that ADMA accumulates in chronic renal failure. Although there is controversy concerning the absolute concentration of ADMA, all authors found a two- to sixfold increase in ADMA levels in patients in chronic renal failure as compared to controls. Different dialysis treatment strategies differentially affect ADMA levels. The presence of atherosclerosis is associated with higher ADMA levels in patients with normal renal function as well as in dialysis patients, but this phenomenon may be unrelated to renal handling of ADMA. Reduced NO elaboration secondary to accumulation of ADMA may be an important pathogenic factor for atherosclerosis in chronic renal failure and ADMA may be a new uremic toxin. Clinical studies on the effect of ADMA are needed to further elucidate its pathophysiological role in atherosclerosis and uremia.

Endothelium-derived nitric oxide (NO) is a potent vasodilator that plays a critical role in regulating vascular resistance and flow [1]. Furthermore, NO inhibits key processes in atherosclerosis, such as monocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation [2]. NO is synthesized by stereospecific oxidation of the terminal guanidino nitrogen of the amino acid L-arginine [3], by the action of a family of NO synthases (NOS) with endothelial, neuroneal and macrophage isoforms [4]. The synthesis of NO can be selectively inhibited by guanidino-substituted analogs of L-arginine like N-monomethyl-L-arginine, which act as competitive antagonists at the active site of the enzyme. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS [4, 5]. It is thought to be derived from proteins that have been post-translationally methylated and subsequently hydrolyzed to release ADMA [4, 6]. A number of cell types elaborate ADMA, including human endothelial cells in culture and blood vessels [4, 7, 8]. ADMA and its biologically inactive stereoisomer symmetric dimethylarginine (SDMA) are at least in part eliminated via urinary excretion and are elevated in renal failure [6, 9, 10]. In addition, ADMA but not SDMA is degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA to L-citrulline and dimethylamine [11]. Two isoforms of this enzyme have been characterized and cloned to date [12]. DDAH I predominates in tissues that express neuroneal NOS and DDAH II predominates in tissues expressing endothelial NOS. This strengthens the hypothesis that methylarginine concentration is actively and cell-specifically regulated in NO-generating tissues, and may play a role in modulating NO release.

EFFECTS OF ADMA ON NITRIC OXIDE ELABORATION

Nitric oxide deficiency can be produced experimentally by the administration of substituted L-arginine analogs that function as competitive inhibitors of NOS. Data from several experimental studies suggest that ADMA concentrations in a pathophysiologically high range (3 to 10 μmol/L) significantly inhibit vascular NO formation by NOS in the presence of L-arginine in isolated human blood vessels, cultured macrophages, and in cultured endothelial cells [13, 14]. It has been reported by our group that plasma levels of ADMA are elevated in indi-

Key words: nitric oxide, atherosclerosis, hemodialysis, uremic toxin, end-stage renal disease.

Table 1. ADMA concentrations in controls, patients on peritoneal dialysis, patients on hemodialysis and the reduction of ADMA by hemodialysis

	ADMA μmol/L			
	Controls	Patients on PD	Patients on hemodialysis	Reduction by hemodialysis %
Vallance et al [10]	1.15 ± 0.13 N = 6	—	8.7 ± 0.7 N = 9	40 N = 3
MacAllister et al [25]	0.36 ± 0.09 N = 9	—	0.9 ± 0.1 N = 9	20 N = 6
Anderstam et al [24]	0.36 ± 0.08 N = 7	0.70 ± 0.27 N = 11	0.70 ± 0.27 N = 19	23 N = 12
AlBanchaabouchie et al [26]	0.41 ± 0.09 N = 37	—	0.82 ± 0.16[a] N = 43	—
Kielstein et al [22]	1.0 ± 0.1 N = 37	2.1 ± 0.4 N = 37	6.0 ± 0.5 N = 43	0/65[b] N = 11
Schmidt et al [23]	0.40 ± 0.08 N = 13	—	4.14 ± 0.78 N = 17	65 N = 11

[a] Patients with a creatinine clearance <10 mL/min
[b] 1 hour after dialysis no decrease; 5 hours after hemodialysis decrease by 65% compared to predialysis ADMA concentration

viduals with hypercholesterolemia or atherosclerosis with normal renal function, suggesting that mechanisms other than decreased renal elimination, for example, reduced degradation of ADMA, may contribute to the elevation of ADMA plasma levels in these disease [15]. Recently, Ito et al described a potential mechanism for this phenomenon. They studied the effects of oxidized LDL (oxLDL) or tumor necrosis factor-α (TNF-α) on the accumulation of ADMA by transformed human umbilical vein endothelial cells (ECV304) and on the enzyme dimethylarginine dimethylaminohydrolase (DDAH) [16]. The addition of oxidized LDL or TNF-α to ECV304 significantly increased the level of ADMA in the conditioned medium. The effect of oxLDL or TNF-α was not due to a change in DDAH expression, but rather to reduced DDAH activity. These results suggest that the impaired endothelial vasodilator dysfunction observed in hypercholesterolemia may be due to reduced degradation of ADMA. A very recent study showed that salt loading increased nitrite/nitrate (NOx) levels in patients with essential hypertension, whereas salt restriction increased plasma NOx. These changes in plasma NOx levels were inversely correlated with those in blood pressure and plasma ADMA level after salt loading and restriction [17]. This study suggests that modulation of NO-synthesis by salt intake may be involved in the mechanism of salt sensitivity in human hypertension, presumably via the change in ADMA concentration. This effect sheds new light on the success of dietary salt restriction and reduction of dialysate sodium to control hypertension in maintenance hemodialysis patients [18].

EVIDENCE FOR IMPAIRED NITRIC OXIDE FORMATION IN RENAL INSUFFICIENCY

In 1992, Vallance et al hypothesized that inhibition of vascular NO formation caused by accumulated ADMA might be responsible for cardiovascular disorders such as hypertension and atherosclerosis, which are frequently observed in end-stage renal disease (ESRD) [10]. Since then the presence of dysfunctional endothelium-dependent vasodilation in this disease has indeed been reported in several clinical investigations. Joannides et al showed that flow induced, NO-dependent forearm vasodilation was impaired in adult patients receiving hemodialysis [19]. Kari et al found impaired flow induced NO-dependent vasodilation in children with chronic renal failure [20]. This was associated with elevated ADMA levels and reduced levels of nitrosothiols in plasma. Moreover, Hand et al demonstrated that the defective endothelium-dependent vasodilation that was present in ESRD-patients before HD was reversed after HD sessions [21]. Furthermore, L-arginine, but not D-arginine, restored endothelial function independently of HD. This study strongly supports the hypothesis that levels of ADMA present in the plasma of ESRD patients induce clinically relevant inhibition of endothelial NO synthase by ADMA, which can be overcome by excess L-arginine [11]. Recently two studies in larger numbers of dialysis patients showed that nitrate and nitrite, the stable inert oxidation products of NO, are decreased [22, 23]. This decrease in NO metabolite concentrations was accompanied by increased plasma ADMA concentrations [22, 23]. In these studies, the concentration of L-arginine, the substrate for NO synthase was not reduced.

ADMA IN RENAL INSUFFICIENCY

Vallance et al were the first to report elevated plasma levels of ADMA and SDMA in a small group of patients with ESRD [10]. In their study dimethylarginine (DMA) levels were elevated about sixfold compared to healthy controls. They suggested that the high incidence of conditions like hypertension, atherosclerosis, and immune dys-

Table 2. L-arginine and nitrite/nitrate concentrations in controls and patients on hemodialysis

	L-arginine concentrations μmol/L		NOx	
	Controls	Patients on HD	Controls	Patients on HD
MacAllister et al [25]	75.3 ± 13 N = 9	35.4 ± 3.1 N = 6	—	—
Kielstein et al [22]	75.5 ± 3.9 N = 37	75.9 ± 7.2 N = 43	39.1 ± 1.9 μmol/L N = 37	23.9 ± 1.7[a] μmol/L N = 43
Schmidt et al [23]	84 ± 11 N = 13	77 ± 7 N = 17	824 ± 96 μmol/L/24 hours N = 13	552 ± 51 μmol/L/24 hours N = 17

[a] $P < 0.05$ vs. controls

function in ESRD patients might be caused at least in part by dysfunction of the L-arginine/NO pathway secondary to accumulation of ADMA. The absolute serum concentration of ADMA is still controversial. Studies by MacAllister et al as well as Anderstam et al found ADMA levels in the range of 0.7 to 0.9 μmol/L in hemodialysis patients [24, 25]. AlBanchaabouchie et al found similar ADMA levels in patients with a creatinine clearance <10 mL/min [26]. However, in these studies ADMA levels in controls were also very low (0.36 to 0.40 μmol/L). More recent studies by Schmidt et al and by our group found ADMA plasma concentrations between 4 and 6 μmol/L, which are consistent with the first description by Vallance et al (abstract; Kielstein et al, *J Am Soc Nephrol* 10:191A, 1999) (Table 1) [10, 22, 23, 27]. The relative increase in ADMA concentrations in ESRD are high enough to cause a diminished release or effect of NO, and consequently cause an increased cerebrovascular tone in uremic patients [14].

INFLUENCE OF HEMODIALYSIS ON ADMA LEVELS

Since ADMA is a small substance with a molecular weight of 202 D, it should easily be cleared by hemodialysis. However, both MacAllister et al and Anderstam et al only found about a 20% reduction in ADMA concentration after hemodialysis [24, 25], which was much less than the 40% reduction first reported by Vallance et al in three hemodialysis patients (Table 2) [10]. However, none of these authors reported the exact time point of blood withdrawal with respect to the hemodialysis session. We recently reported that one hour after hemodialysis there was a slight increase in plasma ADMA concentration, which was significant when the concentration of ADMA was expressed in relation to serum creatinine. At five hours after the hemodialysis session, plasma ADMA levels were decreased by 65% [22]. This finding is line with a study by Schmidt et al, who also found a reduction of ADMA levels by 65% after hemodialysis [23]. Using a batch dialysis system (GENIUS®), we could show that the dialyzer clearance of ADMA is much less than expected from its molecular weight (abstract; Kielstein et al, *J Am Soc Nephrol* 10:191A, 1999). This was also reflected by the low content of ADMA in the dialysate after hemodialysis. These results suggest that mechanisms such as binding to plasma proteins or adsorption to the dialyzer membrane are likely to reduce the removal of ADMA. Furthermore, the fact that plasma levels of ADMA were not decreased at the very end of the hemodialysis session despite the presence of measurable removal suggests that redistribution from the cellular pool and/or de novo synthesis of ADMA may occur during hemodialysis.

ADMA IN PERITONEAL DIALYSIS

Nitric oxide production is impaired not only in hemodialysis, but also in peritoneal dialysis [27]. The possible influence of ADMA in this respect is not clear. While Anderstam et al and Schmidt et al found ADMA plasma levels that were two to five times as high as in control subjects [24, 27] we did not find a significant increase of ADMA compared to control subjects. Peritoneal dialysis-treated patients exhibited lower plasma ADMA concentrations than did hemodialysis-treated patients [22]. This difference may be caused by differences in dialytic clearance of ADMA with the two treatment methods or the metabolism of ADMA.

A NOVEL MARKER OF ATHEROSCLEROSIS AND A NEW UREMIC TOXIN

Exposure to risk factors such as hypertension or hypercholesterolemia decreases the bioavailability of endothelium-derived nitric oxide (NO) and impairs endothelium-dependent vasodilation in animal models. Chronically elevated ADMA concentration possibly induces similar pro-atherogenic effects like those observed in these experimental models [28] and in men [29]. Our group has previously reported that ADMA plasma concentrations are elevated two to threefold in patients with peripheral atherosclerosis [15]. This increase, which was associated with a reduced urinary excretion of nitrate and cyclic guanylic acid (GMP), was independent of the presence of impaired renal excretory function. Moreover, ADMA

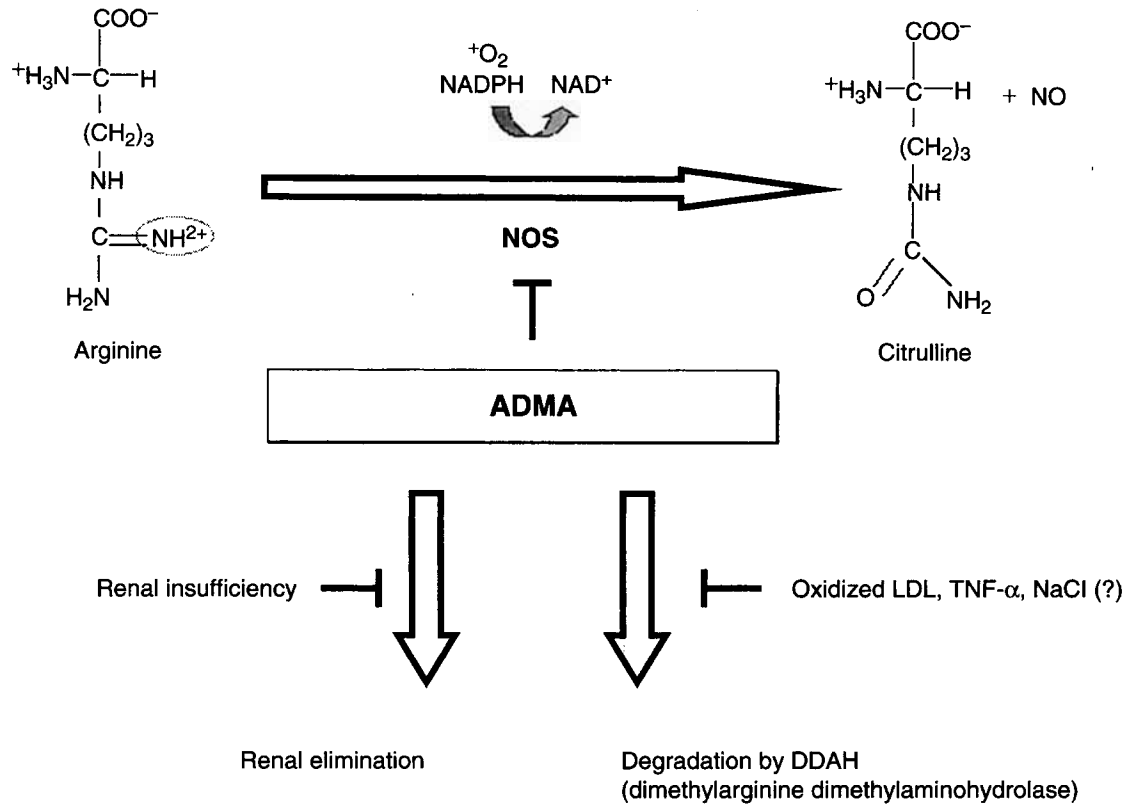

Fig. 1. Accumulation of ADMA in end-stage renal disease (ESRD).

is elevated twofold in the plasma of asymptomatic young hypercholesterolemic subjects with normal renal function [30]. In a recent study we found a similar relative elevation of ADMA levels in ESRD patients with atherosclerosis as compared to ESRD patients without, and in patients with atherosclerosis and normal renal function as compared to controls [22]. A study in 116 subjects revealed that plasma levels of ADMA were positively correlated with age, mean arterial pressure and glucose tolerance. Most intriguingly, stepwise regression analysis revealed that plasma ADMA levels were significantly correlated to the intima-media thickness of the carotid artery as measured by high-resolution ultrasonography [31]. These results suggest that this endogenous antagonist of NO synthase may be a marker of atherosclerosis. It remains unresolved whether ESRD patients with atherosclerosis had higher ADMA levels because of the presence of atherosclerosis, or whether they suffered from atherosclerosis as a consequence of higher ADMA levels. However, the elevation of ADMA levels in atherosclerotic patients with normal renal function as compared to controls suggests that mechanisms other than reduced renal excretion—such as dysregulation of DDAH—may also contribute to the greater accumulation of ADMA in ESRD patients with atherosclerosis as compared to ESRD patients without atherosclerosis. A very recent study showed that plasma levels of ADMA are elevated in hyperhomocyst(e)inemia, an established risk factor of atherosclerosis in ESRD [32]. Disturbed formation and activity of NO may contribute to blood pressure alterations in cardiovascular disease [33]. The concept of accelerated atherosclerosis in chronic dialysis patients has been widely accepted since it was first published by Lindner et al in 1974 [34]. However, many dialysis patients have more or less marked vascular lesions already at the start of dialysis treatment, and the risk factors in the predialysis phase may be of primary importance for the manifestation of cardiovascular disease. Multiple known risk factors have been shown to be present in ESRD patients. Elevated ADMA levels may be a novel preexistent risk factor for atherosclerosis and thereby act as an uremic toxin [22, 35].

In conclusion, it has been well demonstrated that ADMA accumulates in chronic renal failure. Although there is controversy concerning the absolute concentration of ADMA, all authors found a two- to sixfold increase in ADMA levels in patients in chronic renal failure as compared to controls. Different dialysis treatment strategies differentially affect ADMA levels. The presence of atherosclerosis is associated with higher ADMA levels in patients with normal renal function as well as in dialysis patients, but this phenomenon may be unre-

lated to renal handling of ADMA. Reduced NO elaboration secondary to accumulation of ADMA may be an important pathogenic factor for atherosclerosis in chronic renal failure and a new uremic toxin.

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