DIMETHYLARGININE – BIOMARKERS IN PROGRESSION OF KIDNEY DISEASE

DIMETILARGININI – BIOMARKERI U PROGRESIJI BUBREŽNIH OBOLJENJA

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Summary: Decreased nitric oxide (NO) production and/or impaired NO bioavailability may occur in patients with the chronic kidney disease (CKD), and could contribute to elevation of blood pressure, cardiovascular disease (CVD) and progression of renal injury in these patients. Free guanidino-methylated arginine residues occur endogenously as a result of proteolysis of post-translational methylated tissue proteins. The asymmetric dimethyl arginine (ADMA) is a competitive inhibitor of the nitric oxide synthase (NOS) enzymes. The kidney has a predominant role in ADMA elimination by combining two mechanisms; urinary excretion and metabolization of ADMA The degradation of ADMA is accomplished intracellularly by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). ADMA is not only a uremic toxin, but also a strong marker of the endothelial dysfunction and atherosclerosis and a stronger independent predictor of all-cause mortality and cardiovascular outcome in patients with the chronic renal failure. There are at least four mechanisms that may explain the accumulation of ADMA in CKD: increased methylation of proteins, increased protein turnover, decreased metabolism by DDAH and impaired renal excretion. A strong positive correlation between symmetric dimethyl arginine (SDMA) and creatinine suggests that SDMA might be of value as a marker of the renal function. Reduced NO elaboration secondary to accumulation of ADMA and elevated inflammation may be important pathogenic factors for endothelial dysfunction in patients with the renal disease. Elevation of ADMA may be a missing link between CVD and CKD.

Keywords: nitric oxide (NO), asymmetric dimethyl arginine (ADMA), kidney disease, endothelial dysfunction

Kratak sadr`aj: Smanjenje koncentracije NO i/ili nedovoljna raspoloživost ovog molekula kod pacijenata sa bubrežnim bolestima može biti razlog povećanja krvnog pritiska, kardiovaskularnih bolesti (KVS) i progresije bubrežnog oštećenja. Metilarginini nastaju u procesu proteolize post-translaciono metilisanih argininskih rezidua u proteinima. Asimetrični dimetilarginin (ADMA) je kompetitivni inhibitor azot oksid sintaze (NOS). Najvažnija uloga bubrega u eliminaciji ADMA podrazumeva procese urinarne ekskrecije i razgradnju pod uticajem dimetilarginin dimetilaminohidrolaze (DDAH). Na aktivnost DDAH utiču oksidativni stres i inflamacija. ADMA nije samo uremijski toksin već i značajan marker endotelne disfunkcije i ateroskleroze, kao i nezavisni prediktor mortaliteta i kardiovaskularnih bolesti kod pacijenata sa HBI. Osnovni uzroci koji dovode do akumulacije ADMA su povećana metilacija proteina, njihov povećan metabolizam, smanjena aktivnost DDAH i smanjena urinarna ekskrecija. Klirens simetričnog dimetilarginina (SDMA) u plazmi zavisi samo od renalne funkcije (pozitivna korelacija sa kreatininom) i njena akumulacija predstavlja nespecifični indikator uremijskih toksina. Redukovana količina NO praćena akumulacijom ADMA, udružena sa inflamacijom može biti važan patogeni faktor endotelne disfunkcije kod bubrežnih pacijenata. Porast koncentracije ADMA može biti veza između KVS i HBI.

Klju~ne re~i: azot monoksid (NO), asimetrični dimetil arginin (ADMA), bubrežne bolesti, endotelna disfunkcija

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Introduction

Patients with chronic kidney disease (CKD) represent an important segment of the population (7–10%) and, mostly because of high risk of the cardiovascular complications associated with the renal insufficiency, detection and treatment of CKD is now a public health priority. Despite constant improvements in dialysis technology and renal care, the mortality rate is still high in hemodialysis patients. Patients with the end-stage renal disease (ESRD) have 10–20 fold higher cardiovascular disease (CVD) mortality than patients in general population (1–6). These circumstances have led to the research of traditional as well as nontraditional risk factors as potential predictors of mortality in patients on dialysis. Among numerous risk factors, the inhibitors of the nitric-oxide (NO) synthesis deserve special attention, because ESRD patients are characterized by the accelerated atherosclerosis (7). Asymmetric-dimethylarginine (ADMA) is derived largely from the degradation of proteins containing methylated arginine residues and it has been recognized as unique endogenous competitive inhibitor of the nitric-oxide synthase. Approximately 20% of ADMA is cleared by the kidney, whereas the residual is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH).

NO and endothelial dysfunction

The endothelium plays a crucial role in the maintenance of vascular tone and structure. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO), which is formed from amino acid precursor L-arginine by nitric oxide synthase (NOS) (8). NO is the most potent endogenous vasodilator known, exerting its effect via stimulation of soluble guanylate cyclase to produce cyclic GMP (9). NO is a critical modulator of blood flow and blood pressure (10). NO is involved in a wide variety of regulatory mechanisms of the cardiovascular system (CV), including vascular tone (i.e., it is the major mediator of the endothelium dependent vasodilation) and vascular structure (e.g., inhibition of the smooth muscle cell proliferation), and cell-cell interactions in blood vessels (e.g., inhibition of the platelet adhesion and aggregation, inhibition of the monocyte adhesion).

NO has been summarized as an endogenous antiatherosclerotic molecule. Atherosclerosis is a process initiating in the endothelium which may be caused by several vasculotoxic factors. Traditional (Framingham) risk factors like age, sex, smoking, left ventricular hypertrophy (LVH), dyslipidemia and diabetes undoubtedly give an important contribution to high CV mortality of dialysis patients (11). In recent years, much attention has been focused on factors peculiar to end-stage renal disease (ESRD), like anemia, hypoalbuminemia, hyperparathyroidism and hyperphosphatemia, and some emerging risk factors like inflammation and hyperhomocysteinemia (12).

A chronic deficiency or loss of NO activity may contribute to medial thickening and/or myointimal hyperplasia (13). A loss of NO activity occurs early in the course of human vascular disease (14) and it is a contributing factor to abnormal vasomotion and ischemic symptoms. In addition, there is an accumulating evidence that NO deficit participates in the initiation and progression of atherosclerosis.

ADMA: synthesis and elimination

Methylarginines constitute a class of substances formed by posttranslation methylation of the arginine residues in proteins which are subsequently liberated in biological fluids following proteolysis (15). Some of them like endogenous inhibitor of NOS, asymmetric dimethylarginine (ADMA) and L-NG-monomethyl arginine (LNMA) compete with L-arginine for the active sites of this enzyme, while symmetric dimethylarginine (SDMA) can impair NO bioavailability by competing with L-arginine for cellular uptake by y+ transporter for cationic amino acids (16). L-Arginine metabolism is complex and tightly controlled (17).

Proteins that have been posttranslationally methylated and subsequently hydrolyzed are found largely in the nucleolus and appear to be involved in RNA processing and transcriptional control (18). Protein-arginine methyltransferases (PRMTs) catalyze the formation of methylarginine residues (19, 20). These enzymes are classified into type I (PRMT1, PRMT3, PRMT4, PRMT6 and PRMT8) and type II (PRMT5, PRMT7 and FBXO10). Type I PRMTs produces ADMA, while type II PRMTs produces SDMA (21). PRMT activity is influenced by the oxidized lipoproteins *in vitro* (22), and PRMT I expression in endothelial cells and, thereby ADMA level increases in response to shear stress (23).

After proteolytic degradation of methylated proteins, NMA, SDMA or ADMA are released and in part cleared by renal excretion (24). In addition, ADMA, but not SDMA, is degraded in liver, kidney and other organs into citrulline and dimethylamines by dimethylarginine dimethylaminohydrolases (DDAH) (25). It has been estimated that in humans, approximately 300 mol of ADMA is generated per day; approximately 250 μmol of which is metabolized by DDAH, whereas only a minor amount is excreted unchanged by the kidneys (26). This cellular pathway disposes over 2/3 of daily ADMA production, while SDMA is not a substrate for DDAH and it is eliminated almost exclusively via renal route (24). Because ADMA levels are elevated in patients with ESRD, renal excretion of ADMA was considered to be the main route of elimination (27). The kidney has a predominant role in ADMA elimination by combining two mechanisms; urinary excretion and metabolization of ADMA.

DDAH – key enzyme in ADMA level

The central role of DDAH in regulating plasma ADMA levels was shown by using the DDAH inhibitor. Pharmacological inhibition of DDAH activity with S-2-amino-4 (3-methylguanidino) butanoic acid causes ADMA accumulation and thereby induces dose-dependent vasoconstriction of the isolated vascular rings *in vitro* that could be reversed by the addition of L-arginine (28). Degradation of ADMA by DDAH probably involves a nucleophilic attack on the guanidino portion of the molecule by a cysteine held in an activated state in the tertiary structure of the enzyme (29). Many factors, such as oxidized low-density lipoprotein cholesterol, inflammatory cytokines, hyper-homocysteinemia, hyperglycemia, infectious agents, and high doses of erythropoietin, have been shown to attenuate DDAH activity, allowing ADMA to accumulate and block NO synthesis (30).

The finding that DDAH and NOS are co-localized in endothelial cells within the glomerulus and in renal tubular cells supports the hypothesis that the intracellular ADMA concentration is actively regulated in NO generating endothelial cells within the kidney as well (25, 31). Two isoforms of DDAH have been characterized and cloned: DDAH I is found in tissues that express neuronal NOS, whereas DDAH II is found in tissues that express endothelial NOS (32). DDAH I is encoded by genes on chromosome 1, and DDAH 2, by genes on chromosome 6 (33).

The role of ADMA

Data from several experimental studies suggest that ADMA concentrations in a pathophysiologically high range (between 2 and 10 mmol/L) significantly inhibit vascular NO production (34).

Furthermore, ADMA competes (to a lesser degree than SDMA) for L-arginine transport mediated by human cationic amino acid transporter -2B into cells, resulting in L-arginine depletion (35). However, pharmacological ADMA concentrations are necessary to exert this effect *in vitro* (36). A broad range of plasma ADMA levels in pathophysiological situations is one reason why the possible role of ADMA in cardiovascular disease is still considered controversial (37).

Acute systemic administration of a suppressor dose of the ADMA to healthy subjects decreased NO generation, renal perfusion, and sodium excretion without affecting the renin-angiotensin system and sympathetic activity.

ADMA decreases effective renal plasma flow and increases renovascular resistance in a dose-related manner. Moreover, administration of ADMA causes significant sodium retention and blood pressure increase.

The role of SDMA

The ADMA enantiomer SDMA is cleared from the circulation almost exclusively by the kidney and it is a strong marker of the GFR (38). SDMA failed to predict death and clinical outcomes in the sole study testing the relationship between this methylarginine and survival in ESRD patients (39). Experimental studies indicate that SDMA competes with L-arginine for the cell transport system which dose-dependently inhibits NO synthesis (35). On the other hand, elevated SDMA correlates better with organ failure than ADMA in the intensive care patients (40) and SDMA was shown to add risk prediction in patients with low ADMA concentration (41). Plasma SDMA clearance depends mainly on renal function (38) and, therefore, accumulation of this substance is an unspecific indicator of the uremic toxins accumulation, suggesting that the Hb–SDMA association may reflect the influence of uremic toxins on Hb levels.

ADMA and kidney

Malnutrition and enhanced protein turnover, i.e. two main drivers for the enzymes that synthesize ADMA and SDMA (42) are common in ESRD and these alterations are associated with the oxidative stress (43), a factor which inhibits DDAH and hence ADMA degradation in ESRD. The ADMA–SDMA link in ESRD reflects the combined effect of a shared stimulus for biosynthesis (protein degradation) and of markedly impaired removal due either to oxidative stress (ADMA) or abolished renal function (SDMA). Due to its much higher concentration in plasma and biological fluids in renal failure, SDMA is a stronger competitor than ADMA for L-arginine entry into the cell and hence a stronger factor limiting the intracellular availability of this amino acid for NO synthesis. However, ADMA but not SDMA, was associated with the circulating L-arginine levels in ESRD patients. This finding, which specifically replicates in the dialysis population with previous observations in young general population (44) and in essential hypertension (45), may underlie a regulatory mechanism.

In vivo, supplementing L-arginine and thereby increasing the L-arginine/ADMA ratio, a key determinant of NOS activity (46), has been shown repeatedly to increase NO production. This phenomenon has been named the L-arginine paradox (47). In patients with the uremia, this seems to be even more important, because urea inhibits the cell L-arginine transporter *in vitro* at concentrations commonly observed in uremic patients (48). Recent advent of gene-manipulated mice either overexpressing (31) or deleting DDAH (49) will help to further elucidate the pathophysiologic role of ADMA in renal disease and will hopefully help to generate new opportunities for intervention in renal disease progression. Based on the generation and metabolism of ADMA, elevated

levels are the consequence of the increased synthesis (enhanced activity or expression of PRMTs), reduced renal clearance or reduced enzymatic degradation (decreased activity or expression of DDAH). The latter two mechanisms have been shown to contribute to elevations of ADMA in renal disease whereas the role of PRMT under this condition remains unknown.

ADMA, inflammation and kidney

Chronic kidney disease (CKD) has gained much attention as a major health problem. Studies involving CKD patients on maintenance dialysis have shown that these patients are also subjects to chronic inflammation (50). This situation is evident with the increases of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), which are also involved in the pathogenesis of the uremic cachexia (51, 52). In addition, loss of appetite and malnutrition are not rare in these patients, presenting with the increased inflammatory activity. It has been reported that both CKD and inflammation give rise to endothelial dysfunction through the increased levels of the asymmetric dimethylarginine (ADMA) (53). The additive effect of dialysis therapy on ongoing inflammatory and oxidative state has also been demonstrated (54).

Elevated plasma CRP levels positively correlated with the plasma urea levels in HD patients. This finding suggests that the uremia itself might be associated with an elevated inflammation which may contribute to development and progression of atherosclerosis. CRP and ADMA may emerge as important risk factors for atherosclerosis in dialysis patients. Reduced NO elaboration secondary to accumulation of ADMA and elevated inflammation may be important pathogenic factors for endothelial dysfunction in dialysis treatment strategies. However, we suppose that the correlation between activated acute phase and mortality will be strengthened by using two or more measurements of CRP over time. Patients whose CRP levels remain elevated over time would be expected to have an even higher mortality than patients with the occasionally elevated CRP levels (55).

ADMA as cardiovascular risk factor in patients with ESRD

Depending on age, patients on renal replacement therapy encounter 5- to 500-fold higher risk of dying from cardiovascular events. The overall production of nitric oxide (NO) is decreased in chronic kidney disease (CKD), which contributes to cardiovascular events and further progression of kidney damage (56). There are many likely causes of NO deficiency in CKD and the areas surveyed are:

1. Limitations on substrate (L-arginine) availability, probably due to impaired renal L-arginine biosynthesis, decreased transport of L-arginine into endothelial cells and possible competition between NOS and competing metabolic pathways, such as arginase.

2. Increased circulating levels of endogenous NO synthase (NOS) inhibitors, in particular asymmetric dimethylarginine (ADMA). There are at least four possible mechanisms that may explain the accumulation of ADMA in CKD: (i) increased methylation of proteins; (ii) increased protein turnover; (iii) decreased metabolism by DDAH; and (iv) impaired renal excretion.

Observations by Kielstein et al (57) in 1999 refreshed the interest in the potential role of ADMA in accelerated atherosclerosis in patients with ESRD. This cross-sectional study was the first to document that ADMA levels are higher in dialysis patients with than without cardiovascular complications. It is well documented that plasma ADMA concentration is associated with the carotid intima-thickness (58, 59), left ventricular hypertrophy (60), and cardiovascular complications. ADMA plasma concentration was much higher in patients with concentric LVH than in those with eccentric LVH or normal LV mass (61, 62). Several studies have confirmed that ADMA is an independent predictor of all-cause mortality and CVD mortality in patients with ESRD (63) and peripheral arterial disease (64). Very recently, it was reported that the circulating levels of ADMA correlate independently with measures of disease severity and major adverse cardiovascular events (65).

Our data shows that ADMA is an independent and better marker of all-cause and cardiovascular mortality than CRP. This 14-month follow-up study indicates that ADMA is a stronger predictor of all-cause mortality than SDMA and CRP in patients with the end-stage renal disease (66).

ADMA and transplantation

Posttransplant cardiovascular mortality is still an important problem in renal transplant patients. Dimethylarginine metabolism is also of outstanding interest in the context of transplantation, predominantly because the NOS isoforms, endothelial NOS (eNOS) and inducible NOS (iNOS), play protective and deleterious roles in the acute and chronic allograft rejection (67–69). An important hallmark of the chronic rejection of kidneys is the development of allograft vasculopathy, a severe intimal hyperplasia of the renal arteries. The results indicate that elevated plasma level of ADMA is associated with the increased morbidity, mortality, and the deterioration of graft function in renal transplant recipients (70). Transplanted kidneys are prone to oxidative stress-mediated injury by pre-transplant and post-transplant conditions that cause reperfusion injury or imbalance

between the oxidants and antioxidants. Oxidative stress can also be caused by the immunosuppressive therapy. Our findings suggest that renal transplant recipients display persistent oxidative stress. No significant differences in the oxidative stress parameters were found in respect to treatment (71).

Clinical and experimental data evidenced that dimethylarginines contribute to cardiac allograft vasculopathy (72), which resembles vasculopathy of renal allografts (73). Dimethylarginines may play a role in triggering the chronic rejection, but a contribution to the process of vascular remodelling itself is improbable. In contrast, differential arginine methylation by PRMT1 may be involved in pathogenesis of the acute and chronic rejection. When increased soon after transplantation, ADMA may be associated with the episodes of the acute rejection in kidney transplant recipients. The presence of an elevated systolic blood pressure, as well as CRP and ADMA levels, suggested a role for endothelial dysfunction in the development of the acute rejection episodes among deceased donor kidney transplant recipients (74). Shortly after renal transplantation and recipient nephrectomy, ADMA and SDMA are increased, even in the absence of the acute rejection. Cardonuel et al. (75) demonstrated *in vitro* that the L-arg/dimethylarginine ratio must at least decrease to 10 to elicit the physiological effect, and experiments *in vivo* demonstrated that elevated ADMA levels do not necessarily result in the impaired NO production (76). These data suggest that DDAH function is restored within 4 days. We conclude that changes in ADMA levels are not due to impaired renal degradation. DDAH activity, however, may change in other organs, such as liver.

Methylarginine as uremic toxin

ADMA fulfills many of the characteristic features of the uremic toxin. It is a guanidino compound, a product of protein metabolism, accumulates in renal failure, is removed by dialysis, and has a clear mechanism of action (inhibition of NO generation) to produce pathophysiology. However, a predictive power of cystatin C, creatinine, and symmetric dimethylarginine (SDMA) is probably better than that of ADMA. SDMA is not metabolized to any great extent and, as expected, its concentration in plasma correlates closely with creatinine (77, 78).

Conclusion

CRP and ADMA may emerge as important risk factors of atherosclerosis in dialysis patients. Reduced NO elaboration secondary to accumulation of ADMA and elevated inflammation may be important pathogenic factors of endothelial dysfunction in dialysis treatment strategies. ADMA is significantly associated with the progression in patients with mild to moderate kidney disease. Lowering plasma ADMA concentrations, therefore, may represent a novel therapeutic target for prevention of progressive renal damage.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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