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PERSONALIZED MEDICINE: ROLE OF ASYMMETRIC DIMETHYLARGININE AS A PREDICTIVE MARKER OF CAD

S. Mohd. Shiraz Rizvi, Farzana Mahdi, Abbas Ali Mahdi, Tabrez Jafar, Saliha Rizvi

Department of Biochemistry, Department of Molecular and Personalized Medicine

Era's Lucknow Medical College & Hospital, Sarfarazganj, Lucknow, U.P., India-226003

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Address for correspondence

Dr. Saliha Rizvi
Department of Molecular and Personalized Medicine
Era's Lucknow Medical College & Hospital, Lucknow-226003
Email: rizvi_saliha@rediffmail.com
Contact no: +91-9889197879

ABSTRACT

The presence of elevated levels of asymmetric dimethylarginine (ADMA) is concerned with the development of endothelial dysfunction. Free ADMA, which is produced during proteolysis, is actively degraded by the intracellular enzyme dimethylarginine dimethylaminohydrolase (DDAH) which catalyzes the conversion of ADMA to citrulline and dimethylamine. It has been found that more than 70% of ADMA is metabolized by DDAH1. Decreased DDAH expression/activity is evident in disease states related with endothelial dysfunction and is believed to be the mechanism responsible for increased methylarginines which subsequently leads to ADMA mediated eNOS impairment. However, recent studies propose that DDAH may regulate eNOS activity and endothelial function through both ADMA dependent and independent mechanisms. Therefore, elevated plasma ADMA may serve as a marker of impaired methylarginine metabolism and the pathology previously attributed to elevated ADMA may be manifested, at least in part, through altered activity of the enzymes involved in ADMA regulation, specifically DDAH and PRMT.

KEYWORDS: ADMA, Endothelial dysfunction, Nitric Oxide, CVD.

INTRODUCTION

Endothelium derived Nitric Oxide (NO) is a potent vasodilator that plays a significant role in maintaining vascular homeostasis through its anti-atherogenic and anti-proliferative effects on the vascular wall (1). Altered NO biosynthesis has been associated with the pathogenesis of cardiovascular disease and evidence from animal models and clinical studies suggest that accumulation of the endogenous nitric oxide synthase (NOS) inhibitors, asymmetric dimethylarginine (ADMA) and NG-monomethylarginine (NMMA) contribute to the reduced NO generation and disease pathogenesis (2). ADMA and L-NMMA are produced from the proteolysis of methylated arginine residues on various proteins. The methylation is carried out by a group of enzymes which are known as protein-arginine methyl transferase's (PRMT's) (3). Protein arginine methylation has been identified as an significant post-translational modification involved in the regulation of DNA transcription, protein function and cell signaling (4). Upon proteolysis of methylated proteins, free methylarginines are formed which can then be metabolized to citrulline through the activity of Dimethylarginine Dimethylamino Hydrolase (DDAH). Decreased DDAH expression/activity is found in disease states related with endothelial dysfunction and is believed to be the mechanism responsible for increased methylarginines and subsequent ADMA mediated eNOS impairment. Currently there are two known isoforms of DDAH each having different tissue specificity (3). DDAH-1 is thought to be associated with tissues that express high levels of Neuronal Nitric Oxide (nNOS), while DDAH-2 is thought to be associated with tissues that express eNOS (5). However, the biochemical properties and the contribution of each enzyme to the regulation of endothelial NO production has yet to be elucidated (6).

Intact healthy endothelium plays an important role in vascular homeostasis which releases number of regulatory substances to maintain the vascular tone. Damaged endothelium cannot perform this crucial task and it becomes dysfunctional which is the initial event on the long path towards atherosclerosis (7). Among these regulatory substances Nitric Oxide (NO) is most important vasodilator substance produced by the endothelium (3-7) which also inhibits the adhesion and aggregation of platelets, adhesion of monocytes and leukocytes to the endothelium, vascular smooth muscle cell proliferation and low density lipoprotein (LDL) oxidation (8). A bridged NO bioavailability affects all

these functions which leads to initiation and progression of atherosclerosis (9). Nitric oxide, an 'endogenous anti-atherogenic molecule', is produced from L-arginine by endothelial Nitric Oxide synthase (eNOS) which requires molecular oxygen and various cofactors. Any disease state which decreases endothelial NO production possibly will consequently promote atherosclerosis (10).

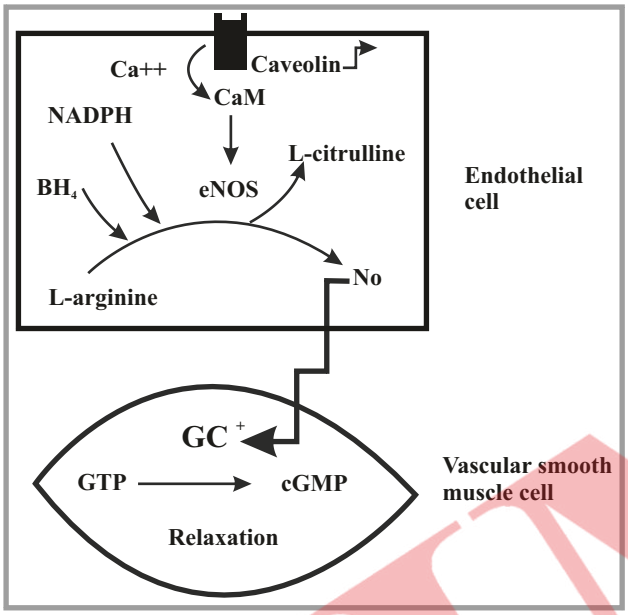

Fig 1: Production of NO by endothelial cells (11)

ADMA, being structural analogue of L-arginine functions as an endogenous competitive inhibitor of eNOS and it contributes to endothelial dysfunction (12). A number of studies have revealed that high level of baseline ADMA is independently prognostic of adverse outcomes in a variety of populations including stable angina, unstable angina and established coronary artery disease (13). However, very less is known about the relationship of serum ADMA and percentage of the atherosclerotic block in Coronary Artery Disease (CAD) patients. Considering the promising role of ADMA & NO in cardiovascular diseases it is found that serum ADMA/NO ratio in CAD patients can assess the severity (percentage) of atherosclerotic block (14).

BIOSYNTHESIS OF ADMA, IT'S METABOLISM AND EXCRETION

ADMA Biosynthesis

Degradation of methylated proteins leads to the formation of Dimethylarginines (15). With the participation of the enzymes protein arginine methyl transferase type 1 and 2 (PRMT1, PRMT2), the methyl groups are obtained from S-adenosylmethionine (16). PRMT-1 catalyses the production of NGmonomethyl-L-arginine (LNMMA) and NG, NG-dimethyl-L-arginine (ADMA), whereas the release of NG, NG-dimethyl-L-arginine (symmetric dimethylarginine; SDMA) and L-NMMA is through methylation of proteins by PRMT-2 (17). The asymmetrically methylated arginine residues (L-NMMA and ADMA), are competitive inhibitors of the nitric oxide synthases and symmetrically methylated arginine (SDMA) do not inhibit it. The formation of ADMA from endothelial cells is raised in the presence of native or oxidized LDL, possibly mediated by up-regulation of S-adenosylmethionine dependent methyl transferases (18) (Fig. 2). Furthermore, it has been obsereved in recent data that the lung appears to contain increased amounts of protein bound ADMA, due to the high expression levels of various PRMTs in lung tissue (19).

Metabolism of ADMA

Hydrolytic degradation to citrulline and dimethylamine catalysed by the enzyme called NG dimethylarginine dimethylaminohydrolase (DDAH) (20) is the specific mechanism involved in metabolism of ADMA but not SDMA. DDAH activity is found in kidney, pancreas, liver, brain and aorta with immunoexpression also in neutrophils and macrophages (21). DDAH inhibition leads to gradual vasoconstriction which is reversed by L-arginine (22). There are two isoforms of DDAH, DDAH-1 and DDAH-2. DDAH-1 is commonly found in tissues expressing neuronal NOS while DDAH-2 is predominantly present in tissues containing the endothelial isoform of NOS (23). Raised plasma levels of glucose, oxidized LDL and homocysteine are related with reduced levels of DDAH. Moreover, a few conventional cardiovascular risk factors may decrease DDAH activity by increasing oxidative stress (24-25). Pharmacological inhibition of DDAH leads to increase in ADMA concentrations and decreases NO production (26). Conversely, transgenic DDAH over expression decreases ADMA levels and increases NO levels (27). In animal studies over expression of DDAH has been shown to promote endothelial repair after vascular injury, to suppress myocardial reperfusion injury and to inhibit ADMA induced endothelial dysfunction in the cerebral circulation (28). Moreover interestingly, over-expression of DDAH-1 and DDAH-2 causes very similar phenotypic changes, whereas selective silencing of individual DDAH isoforms leads to greatly different biological effects (29). Thus, silencing of DDAH-1 resulted in raised circulating ADMA levels but no change in endothelium-mediated vasodilation, whereas silencing of DDAH-2 resulted in significantly decreased endothelium-mediated vasodilatation with no concomitant change in plasma ADMA concentration. These findings suggests that DDAH-2 is the most abundant isoform in endothelium, whereas DDAH-1 is found at high expression levels in kidneys and liver (30).

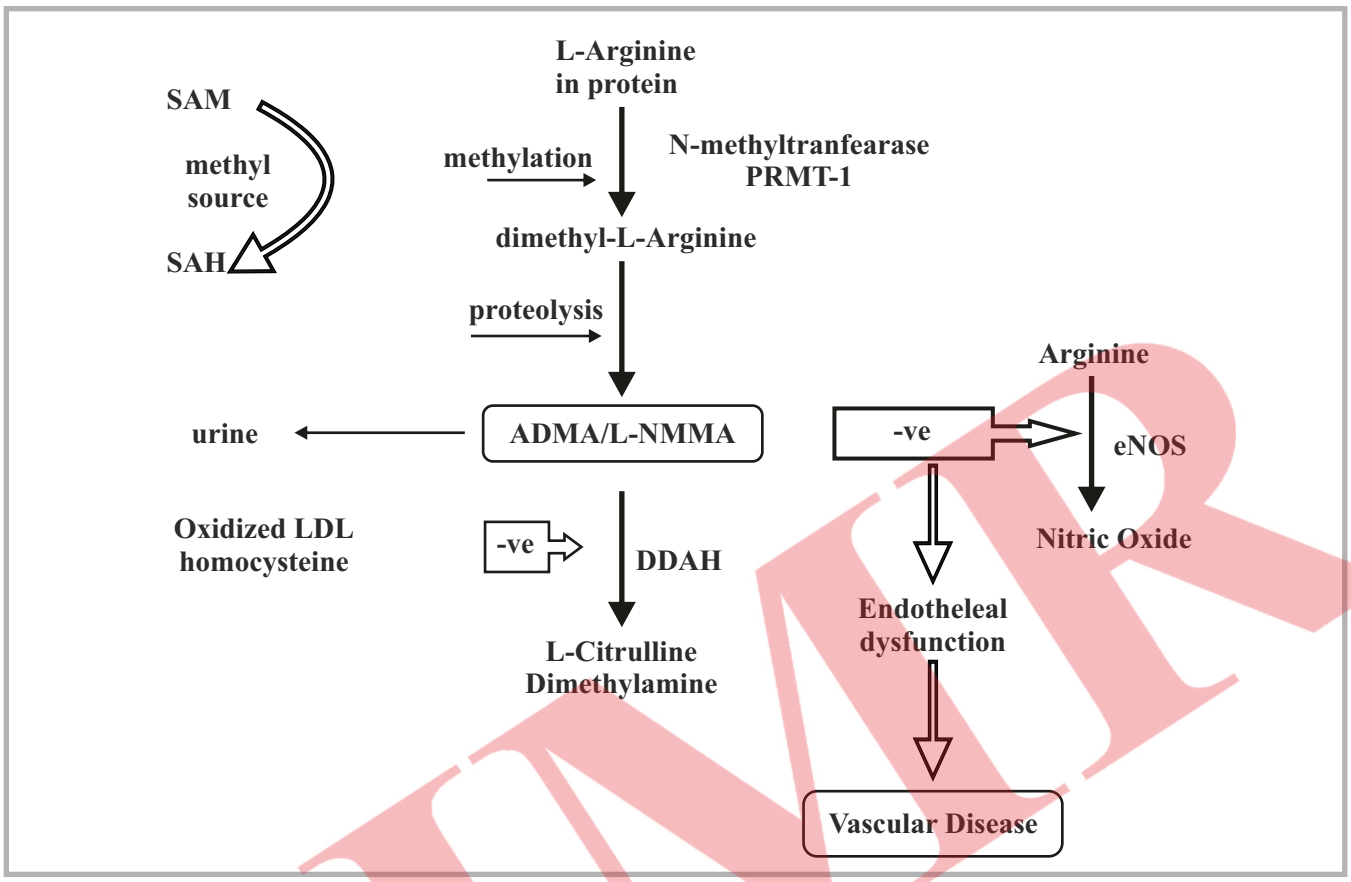

Fig 2: Overview of Pathways of Synthesis and Metabolism of ADMA (31)

Exretion of ADMA

Elimination of endogenous ADMA and SDMA mostly occurs through renal. Urinary excretion of SDMA in rabbits has been shown to be 30 times greater than that of either L-NMMA or ADMA (32). A number of studies have reported raised levels of ADMA and SDMA in people with renal failure (33). Interestingly, haemodialysis causes a lower clearance of ADMA than predicted, suggesting there are alternative non-renal route(s) of removal of circulating ADMA (34).

ENDOTHELIAL DYSFUNCTION AND ADMA

The excretion of methylated arginines occurs in urine and it has since been demonstrated that ADMA antagonizes endothelium-dependent vasodilation, a phenomenon first observed in chronic renal failure (35). ADMA is now acknowledged to be a mediator molecule of the adverse vascular effects of many other factors and markers of cardiovascular risk. ADMA is characterized as an amino acid of intracellular origin naturally found circulating through the plasma, urine, tissues, and cells (36). Its synthesis occurs when arginine residues in the nuclear proteins are methylated through the action of the protein arginine methyltransferases (PRMTs), which are largely distributed throughout the human body, through a posttranslational change that adds one or two methyl groups to the nitrogens of the guanidine incorporated into the proteins (37). Two types of PRMTs are found, with several isoforms: type 1 catalyzes the formation of ADMA, and type 2 catalyzes the formation of the symmetric dimethylarginine (SDMA); but both enzymes can transfer the methyl radical, producing the NGmonomethyl-L-arginine (L-NMMA). Of these, only the asymmetrically methylated species (ADMA and L-NMMA) inhibits NOS; SDMA do not (38). The three isoforms of NOS are inhibited by ADMA which is equipotent with L-NMMA. It can also uncouple the enzyme, produce superoxides, and it interfaces with other targets in the cell. The administration of ADMA in rats causes an increase in the renal vascular resistance and blood pressure, confirming its biological action in vivo. Its levels are much greater intracellularly than extracellularly, sufficient in some cases to inhibit NOS, as demonstrated with cultivated endothelial cells (39). However, an independent additional action modality was demonstrated in vivo, in which the chronic infusion induced vascular injuries in eNOS knockout mice. Additionally, the three methylarginines interfere with the transport of L-

arginine as mediated by the cationic amino acid carrier within the plasma membrane (y+ channels), explaining the SDMA inhibitor effect in the context of NO generation. Renal excretion is partly responsible for the elimination of methylarginines - SDMA is mainly involved, but ADMA and L-NMMA are also extensively metabolized and produce citrulline and dimethylamine by the action of the dimethylarginine dimethylaminohydrolases (DDAHs). This enzymatic group presents in superior organisms two isoforms codified by genes on chromosomes 1 (DDAH-1) and 6 (DDAH-2), with distinct tissue-relevant distributions but seemingly similar activities. The regulation of gene expression and DDAH activity remains highly largely unclear. Incubating endothelial cells with tumor necrosis factor–alpha (TNF-alpha) or oxidized low density lipoprotein (LDL-ox) decreases the activity of this enzyme. Other factors, such as the oxidative stress associated with S-nitrosylation and high levels of glucose and homocysteine, contribute to decreasing DDAH activity, leading to an elevation of ADMA levels (40). Elevated ADMA in the context of renal insufficiency occurs in a variety of ways, probably because the DDAH activity can vary under different situations. This molecule is dialyzable, but it reaches pathological concentrations after dialysis process. In the disease state of chronic renal insufficiency, it fulfills the criteria of an uremic toxin because it increases in inverse proportion to diminishing renal function. It is a guanidine compound, and a product of protein metabolism. Various biological functions can be impacted by inhibiting NOS, including the cardiovascular, osseous, and immune systems. Subsequent research has also suggested the involvement of the liver in the metabolism of dimethylarginines. Various research workers have demonstrated that hepatocytes abundantly express y+ channels in their membranes and contain high concentrations of DDAH (41). Moreover a recent study carried out in patients who underwent hepatic surgery confirmed the entrance of ADMA in the liver. These and other metabolic studies have demonstrated that the liver is essential in regulating plasma concentrations of dimethylarginine.

ADMA AS A MEDIATOR OF CAD

Plasma levels of ADMA have been shown to be raised in diseases associated with endothelial dysfunction including hyperlipidemia, hypertension, diabetes mellitus, and others (42). Furthermore, it has been found that ADMA predicts cardiovascular mortality in patients with coronary heart disease (CHD). Study published recently from the multicenter Coronary Artery Risk Determination investigating the influence of ADMA Concentration (CARDIAC) has indicated that increased plasma levels of ADMA is an independent risk factor for CAD (43). Moreover, whether the increased risk related with raised ADMA levels is a direct result of NOS impairment is an area of debate. Therefore, considerable research about the contribution of ADMA to the regulation of NOS-dependent NO production has been started.

Studies involving DDAH gene silencing techniques and DDAH transgenic mice present a strong evidence for involvement of ADMA in endothelial dysfunction. Tanaka et. al. have demonstrated that DDAH-1 transgenic mice gets protection against cardiac transplant vasculopathy (44). Wang et. al. used in-vivo siRNA techniques and demonstrated that DDAH-1 gene silencing raised plasma levels of ADMA by 50% but this elevation had no effect on endothelial dependent relaxation. In contrast, in-vivo DDAH-2 gene silencing had no effect on plasma levels of ADMA but reduced endothelial dependent relaxation by 40% (45). Therefore, these findings present a higher significance and demonstrate that raised plasma ADMA is not associated with impaired endothelial dependent relaxation while loss of DDAH-2 activity is related with impaired endothelial dependent relaxation, despite the fact the plasma ADMA levels are not elevated.

CONCLUSION

Data from previous studies done on association of ADMA in relation to CAD suggests that there is a significant increase in serum ADMA levels and concomitant decrease in NO levels in patients of CAD. The mechanism involved is due to reduced expression of DDAH activity which leads to desease states associated with endothelial dysfunction and is therefore suggested to be a mechanism which is responsible for raised levels of methylarginines which subsequently causes ADMA mediated eNOS impairment, which further aggravates the atherosclerotic progression increasing the severity of atherosclerotic block in CAD. Therefore, ADMA can be used as a predictive marker of CAD, and also ADMA/NO ratio can be used as a marker to access the severity of CAD.

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