

The effect of pharmacological treatment on ADMA in patients with heart failure

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1. ABSTRACT

Asymmetric dimethylarginine (ADMA) plays a crucial role in the arginine-nitric oxide (NO) pathway. NO plays an important role in controlling vascular tone and regulates the contractile properties of cardiac myocytes. The purpose of this study was to investigate the effect of pharmacological treatment on asymmetrical dimethylarginine (ADMA) plasma levels in patients with acute congestive heart failure (HF). Patients with symptomatic acute congestive HF (NYHA Class III-IV) and impaired left ventricular (LV) function (ejection fraction < 40%) were included in the study. ADMA and SDMA concentrations were assessed before and after pharmacological treatment in 18 critically ill patients on the intensive care unit by high performance liquid chromatography. All patients received a complete pharmacological treatment (diuretics, digoxin, ACE-inhibitors or angiotensin receptor blockers, and nitroglycerin) for the treatment of acute congestive HF. ADMA plasma levels of critically ill patients were significantly higher after pharmacological treatment respect baseline values (pre-treatment). In critically ill patients with acute congestive HF acute renal impairment function and the modulation of NOS determine plasma ADMA/SDMA levels after therapy.

2. INTRODUCTION

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthases (NOS). Apart from ADMA, two related compounds, symmetric dimethylarginine (SDMA) and N-monomethyl-L-arginine (L-NMMA) are synthesized endogenously. L-NMMA is as potent as ADMA in decreasing NOS activity but its concentration in plasma is about tenfold lower (1), indicating that both are important NOS regulators. SDMA at concentrations in the circulation are comparable to ADMA but it has no effect on NOS (2). ADMA, SDMA and L-NMMA are synthesized during the methylation of protein arginine residues by S-adenosylmethionine protein arginine methyltransferases (protein methylases, PRMT). These enzymes transfer the methyl group from S-adenosylmethionine (3,4).

Published experimental data suggest that ADMA released upon hydrolytic breakdown of methylated proteins, is eliminated from the body by the activity of dimethylarginine dimethylaminohydrolase (DDAH), which cleaves ADMA to form L-citrulline and dimethylamine (5) In humans there are two isoforms of DDAH (DDAH-1 and DDAH-2) with partly overlapping tissue distributions in different tissues (6).

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ADMA plasma concentration is elevated in a wide range of conditions associated with an increased risk of cardiovascular disease (CVD), and in patients with established CVD (7). Whilst the molecular mechanisms underlying the elevation of ADMA plasma levels are not yet fully understood for all of these conditions, recent pathophysiological evidence has suggested that oxidative stress, like it is brought about by oxidized LDL cholesterol and by chemokines like tumor necrosis factor- α , may lead to inactivation of DDAH-2 (8).

Moreover recent data demonstrated that some endothelial dysfunction may be related to reduced activity and/or expression of DDAH (9,10).

Heart failure (HF) is a pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the requirements of the metabolizing tissues or can do so only from an elevated filling pressure. HF may be caused by myocyte death, myocyte dysfunction, ventricular remodeling or some combination (11). HF include chronic compensated heart failure (CCHF) and acute decompensated congestive heart failure (ADHF). Acute exacerbation was defined the condition of worsening symptoms, mainly dyspnoea, for which the patients need to be hospitalized. Patients with exacerbation of CCHF have a poor prognosis, with a 60 day mortality rate of almost 10% and frequent rehospitalizations (12,13).

Elevated ADMA plasma concentrations have been described in patients with CCHF in parallel with the severity of disease and in ADHF related to the suppression of the higher level of plasma NO (14-16).

Since CAHF patients need to be hospitalized and undergo to pharmacological treatment including diuretics, digoxin, ACE-inhibitors or angiotensin receptor blockers, and nitroglycerin, the aim of this study was to investigate the effect of pharmacological treatment on ADMA plasma concentrations in patients with ADHF.

3. MATERIALS AND METHODS

3.1. Study design

Between January until March 2010 all consecutive patients with diagnosis of ADHF admitted in Intensive Cardiology Unit of San Camillo De Lellis Hospital (Manfredonia, Italy) were invited to participate in this study. Inclusion criteria were written informed consent, left ventricular ejection fraction (LVEF) of < 40%. Exclusion criteria were acute cardiac decompensation within the previous 7 days, need for coronary revascularization, or acute coronary syndrome, age under 18 or above 75 years, impaired hepatic function (prothrombin time > 1.5 times the upper limit of normal or alanine aminotransferase (ALT) > 2.5 times upper limit of normal). ADHF was defined as acute and progressive resting dyspnoea associated with clinical signs of pulmonary or peripheral congestion requiring hospitalization and treatment with an intravenous diuretic.

3.2. Patients

Eighteen patients with ADHF and impaired LVEF as assessed from echocardiography were eligible for

the study. At study entry, medical and surgical history, physical condition, and medication were recorded. Details of HF diagnosis and history of cardiac congestive HF were evaluated by review of hospital records. After inclusion, a heparinised blood sample was drawn from an indwelling arterial line for determination of ADMA and SDMA at baseline. Subsequently a blood sample was drawn after one day of therapy (diuretics, digoxin, ACE-inhibitors or angiotensin receptor blockers, and nitroglycerin). Simultaneously, laboratory parameters indicating renal function (creatinine, urea) and hepatic function (aspartate aminotransferase (AST), alanine aminotransferase (ALT), complete haematocytometer exam, and blood sugar were determined before and after one day of therapy.

3.3. Sample collection, storage and preparation

Blood samples were collected in polypropylene tubes containing EDTA 1 mM. Samples were stored in an ice box prior to centrifugation at 3000g for 10 min at 4°C. 200 μ l aliquots of plasma were transferred into Eppendorf tubes. Plasma samples were either used for extraction immediately or stored in the dark at -80°C until analysis was performed.

3.4. Biochemical analysis

The concentration of ADMA and SDMA were determined by high-performance liquid chromatography (HPLC) as described previously (17). In brief, solid-phase extraction on polymeric cation-exchange columns was performed after addition of monomethylarginine as the internal standard. After derivatization with ortho-phthalaldehyde reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection. Laboratory parameters indicating liver and renal function, complete haematocytometer exam, and blood sugar were measured by standard methods in the clinical laboratory.

3.5. Statistical analysis

Results were expressed as mean \pm SD. Data were analysed by using SPSS statistical software (version 15.0 for Windows; SPSS Inc., Chicago). For each baseline characteristic, the mean value or the corresponding percent of study participants was calculated. The significance of changes in ADMA and SDMA was examined using the paired Student *t*-test. A two-tailed *p* value <0.05 was considered significant

4. RESULTS

Eighteen patients with ADHF were included in the study. Demographic details are summarized in Table 1. Mean age was 65 \pm 7 years, 9 patients were males (9 females), 10 patients with III NYHA functional class and 8 patients with IV NYHA functional class. Means \pm SD of laboratory data are summarized in Table 2. There was no difference in laboratory parameters indicating renal (creatinine) and hepatic (AST, ALT) functions, complete haematocytometer exam (hemoglobin, red and white cells, haematocrit), and glycaemia, before and after therapy. Moreover, ADMA and SDMA plasma levels were significantly higher in critically ill patients after

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Table 1. Demographic details of population study

Patients characteristics	
Age (mean \pm SD)	65 \pm 7
Sex (M/F)	8/8
BMI (Kg/m ²)	30.15 \pm 2.55
NYHA functional class III	10
NYHA functional class IV	8

Table 2. Laboratory data

	Pre-treatment	Post-treatment	P-value
Creatinine (mg/dL)	1.76	1.68	ns
Urea (mg/dL)	85	87	ns
AST	31	30	ns
ALT	30	32	ns
HGB L(g/d)	12.3	12.2	ns
Red cells	4.206.000	4.190.000	ns
White cells	8.780	8.795	ns
Haematocrit	37.18	37.48	ns
Glycaemia	90	88	ns
LVEF (%)	34	39	p<0.01
ADMA (μ mol/L)	0.66 \pm 0.22	0.75 \pm 0.26	p<0.01
SDMA (μ mol/L)	1.22 \pm 0.35	1.34 \pm 0.37	p<0.01

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pharmacological treatment (ADMA 0.75 \pm 0.26 μ mol/L; SDMA 1.34 \pm 0.37 μ mol/L) compared to basal (pre-treatment) levels (ADMA 0.66 \pm 0.22 μ mol/L; $p < 0.01$, SDMA 1.22 \pm 0.35; $p < 0.01$ respectively).

7. DISCUSSION

In our study we have considered patients with ADHF, a syndrome manifesting as the inability of the heart to fill with or eject blood due to any structural or functional cardiac conditions (18), and responsible for more hospitalizations than all forms of cancer combined (19). The common pathophysiologic state that acute HF is extremely complex. Compensatory mechanisms exist on every level of organization from sub-cellular all the way through organ-to-organ interactions. Most important among these adaptations are the (a) Frank-Starling mechanism, in which an increased preload helps to sustain cardiac performance; (b) alterations in myocyte regeneration and death; (c) myocardial hypertrophy with or without cardiac chamber dilatation, in which the mass of contractile tissue is augmented; and (d) activation of neurohumoral systems, especially the release of norepinephrine by adrenergic cardiac nerves, which augments myocardial contractility and includes activation of the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (SNS), and other neurohumoral adjustments that act to maintain arterial pressure and perfusion of vital organs (20).

In ADHF, the finite adaptive mechanisms that may be adequate to maintain the overall contractile performance of the heart at relatively normal levels become maladaptive when trying to sustain adequate cardiac performance. As HF advances, there is a relative decline in the counterregulatory effects of endogenous vasodilators, including nitric oxide (NO), prostaglandins (PGs), bradykinin (BK), atrial natriuretic peptide (ANP), and B-type natriuretic peptide (BNP). This occurs simultaneously with the increase in vasoconstrictor substances from the RAAS and adrenergic systems (21).

In our study we have found significant high levels of ADMA and SDMA in plasma of ADHF patients compared to post-treatment values. Notably, SDMA, the biologically inactive structural isomer of ADMA, is elevated in parallel with ADMA. This may suggest increased hydrolysis of proteins containing methylated arginines as a results of the increased catabolism that is known in ADHF patients. The increased ADMA/SDMA levels can be explained by: (a) reduction of renal excretion of ADMA/SDMA due to acute renal impairment function as reported by Nijvelet et al. (22,23); (b) increased methylation of proteins by PMRT and impaired metabolism of DDAH due to shear stress that increases PRMT without change in DDAH activity, resulting in enhanced ADMA release as reported by Osanai (24); (c) however, the most common mechanism leading to accumulation of ADMA involves impaired metabolism of DDAH by oxidative stress (7, 25). The higher concentration of ADMA and SDMA demonstrate that in acute HF are impaired the metabolism of PMRT1 and PMRT2 (two isoforms of PMRT) (26).

NO plays an important role in controlling vascular tone and regulates the contractile properties of cardiac myocytes. Patients with HF exhibit high plasma levels of nitrite/nitrate (NOx), a stable metabolite of NO, and of cytokines such as tumor necrosis factor-alpha (TNF- α), a potent inducer of NOS (14). These findings raise the possibility that local or systemic overproduction of NO induced by cytokines exerts a negative effect on the myocardium and may have detrimental effects on systemic hemodynamics in patients with acute HF. ADMA is related to exacerbation of ADHF by suppression of the compensatory higher level of plasma NO (14). Accumulating evidence suggests that ADMA is involved in the pathogenesis of various conditions such as hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, obesity, and hyperhomocysteinemia (10,27-32). Moreover, in these conditions different pharmacological

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treatments were found to improve endothelial function and NO availability (33-36). In our study we have found significant high levels of ADMA and SDMA after treatment of ADHF compared to basal (pre-treatment) values. The patients were been treated with diuretics, digoxin, ACE-inhibitors or angiotensin receptor blockers, and nitroglycerin. Even if any drugs used might improve endothelial function occur at least, four weeks of treatment to significantly decrease plasma ADMA levels (5,37). For this reason, patients with exacerbation of chronic HF, even if received pharmacological treatment, have a poor prognosis, with high mortality rate (12,13). Further studies are needed to better understand the clinical implications of therapeutic interventions targeting these cellular metabolic components such as PRMT, DDAH, or NOS in the setting of ADHF.

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