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ORIGINAL ARTICLE

High clinical accuracy of asymmetric dimethylarginine and symmetric dimethylarginine in patients with ischemic heart disease

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Abstract Elevated plasma concentrations of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) were found in various clinical settings including coronary heart disease. To assess ADMA and SDMA diagnostic validity in patients with different stages of ischemic heart disease, we studied these markers in patients having stable angina pectoris (SAP), unstable angina (USAP), and acute myocardial infarction (AMI). The results were compared with the values of healthy individuals. Plasma ADMA and SDMA levels were measured by high-performance liquid chromatography. In all patient groups both markers were significantly elevated in comparison with control ones ($p < 0.001$). In SAP patients, the median ADMA value was 0.75 (0.31–2.73) μmol/L, and SDMA 1.11 (0.69–0.1.42) μmol/L, in USAP patients, the marker values were 0.94 (0.34–3.13) μmol/L and 1.23 (0.88–4.72) μmol/L, and in AMI patients, 0.98 (0.48–2.01) μmol/L and 1.26 (0.75–2.93) μmol/L, while in healthy subjects they were 0.31 (0.17–0.87) μmol/L and 0.29 (0.20–0.83) μmol/L, respectively. SDMA was found significantly different in SAP and AMI patients ($p < 0.05$). Diagnostic accuracy was determined by receiver operating characteristic (ROC) curve analysis. The highest area under the ROC (AUC) for ADMA was obtained in AMI patients (0.976), while for SDMA in USAP patients (1.000). There was no significant difference between the AUCs. The greatest sensitivity and specificity were found in the USAP group (95.65 and 96.30 % for ADMA, and 100 % for each characteristic of SDMA). Considering these results, SDMA showed better clinical accuracy in assessing ischemic disease, where it could be used as a valid marker and a therapeutic target.

Keywords Asymmetric dimethylarginine · Symmetric dimethylarginine · Ischemic heart disease

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Introduction

In the last decade, scientists have focused mainly on asymmetric dimethylarginine (ADMA), which has been shown to correlate with traditional and non-traditional cardiovascular risk factors (Cooke 2004). Asymmetric dimethylarginine is a natural, competitive inhibitor, and besides the substrate L-arginine, one of the primary factors controlling nitric oxide synthase (NOS) activity. ADMA is produced by the methylation of specific arginine residues of certain cellular proteins mostly found in the nucleus. The methylation produces monomethylarginine and two forms of dimethylarginine, depending on whether the methyl groups are on the same nitrogen atom (ADMA) or two different nitrogen atoms (SDMA-symmetrical dimethylarginine). SDMA has insignificant inhibitory effects on NOS. The

methylation of arginine residues is catalyzed by the enzymes called protein arginine N-methyltransferases (Rust et al. 2011). Both isomers are released from proteins by proteolysis. ADMA is cleared by the hepatic enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Nijveldt et al. 2003) and by the kidney (Wahbi et al. 2001), while SDMA is mainly eliminated by renal excretion (Kielstein et al. 2006).

ADMA was found to be elevated and closely correlated with the impaired vasodilator function in conditions associated with endothelial dysfunction, such as hypercholesterolemia, hypertension, insulin resistance and type 2 diabetes, hyperhomocyst(e)inemia and renal insufficiency (Cooke 2000). However, ADMA also seems to be involved in myocardial ischemia, since its plasma levels predict future coronary events in patients with an elevated cardiovascular risk (Valkonen et al. 2003). Recently, elevations of plasma ADMA concentrations in acute coronary events independent of cardiovascular risk factors were observed (Bae et al. 2005). Plasma ADMA levels are unaffected by statin treatments (Päivä et al. 2003), while ACE inhibitors lower plasma ADMA concentrations (Delles et al. 2002) improve coronary microvascular function and reduce myocardial ischemia (Chen et al. 2002).

In this study, we determined whether ADMA and SDMA plasma concentrations might be valid diagnostic and stratification markers in patients with ischemic heart disease. The clinical accuracy of both markers was assessed by receiver operating characteristic (ROC) curve analysis in patients with stable and unstable angina pectoris, respectively, and acute myocardial infarction (AMI). In addition, we studied the interdependence between plasma ADMA and SDMA concentrations and traditional cardiovascular risk factors.

Materials and methods

Subjects

This study included 74 (48 males, 26 females, mean age 61.6 ± 10.8) patients with stable angina pectoris (SAP), 74 (44/30, 62.5 ± 7.3) with unstable angina pectoris (USAP), 94 (63/31, 62.3 ± 6.8) patients with (AMI), and 66 healthy subjects (30 males, 36 females; mean age 58.7 ± 5.6) as a control group. The patients were selected at the Institute for Cardiovascular Diseases "Niška Banja" and were treated for the evaluation of chest pain. All patients in the SAP group had a history consistent with stable angina for at least 3 months before admission to the Institute, and in all patients myocardial ischemia was established on exercise electrocardiogram and/or stress echocardiogram. None of the patients in this group had previous myocardial infarction or myocardial revascularization, cardiac valve disease, cardiomyopathy, malignant arrhythmias, acute or chronic liver disease, renal failure, or inflammatory disease, and none of the patients was subjected to coronary percutaneous interventions or coronary artery bypass graft operation at the time of the inclusion into the study.

Unstable angina was defined according to Hamm and Braunwald (2000). All patients in the USAP group had chest pain of increasing frequency and severity or at rest during the last 48 h before admission to the Institute without a rise in cardiac enzymes and troponin I. The diagnosis of AMI was based on the following criteria: chest pain persisting longer than 30 min, concomitant changes on the electrocardiogram at the admission to hospital and elevated troponin I levels. All patients were selected according to the Guidelines of the European Society of Cardiology (van de Werf et al. 2003). A detailed history, including data for risk factors and current medications, was performed in all patients just after admission. Additional explanations of the criteria for the selection of patients with ischemic heart disease were given in our previously published paper (Djordjević et al. 2008).

The control group included healthy volunteers—blood bank donors from the Department for Blood Transfusion of the Clinical Centre Niš. All controls were free of any acute infectious disease and they had no history of hypertension, diabetes or ischemic heart disease. All subjects gave informed consent prior to their enrollment in the study, and the study was approved by the local Ethics Committee.

Methods

ADMA and SDMA were analyzed in subjects' blood plasma essentially following the procedures already described (Paroni et al. 2005; Teerlink et al. 2002). Briefly, plasma (0.2 mL), to which monomethylarginine (50 µL, 10 µM) was added as internal standard (IS), was applied to a mixed-mode cation-exchange SPE cartridge (Supelco Discovery® DSC-MCAX, 100 mg/mL, Supelco, Bellefonte, PA, USA) previously activated with methanol (1 mL) and TCA 2 % (2 mL). After the column washing (TCA 2 %, 150 mmol/L phosphate buffer pH 8.0, methanol), the amino acids were eluated with 1.2 mL of 2 % triethylamine (TEA) solution in methanol:water (70:30; v:v). The eluate was dried under nitrogen, redissolved in 0.4 mL starting buffer solution and derivatized with OPA (*ortho*-phthaldialdehyde) reagent (0.1 mL, 2 min) before analysis.

The HPLC analysis was carried out on the instrument Agilent 1200 Series equipped with an autosampler and a binary pump. Compounds were detected with a fluorescence detector at excitation 340 nm and emission 455 nm,

Table 1 Demographic and biochemical characteristics and risk factors in patients with ischemic heart diseases

	AMI ($n = 94$)	USAP ($n = 74$)	SAP ($n = 74$)	Control ($n = 66$)
Age (years)	62.3 ± 6.8	62.5 ± 7.3	61.6 ± 10.8	58.7 ± 5.6
Sex (male/female, n)	63/31	44/30	48/26	30/36
Blood biochemical data				
Glucose (mmol/L)	6.7 ± 2.7**	6.2 ± 2.5**	5.7 ± 1.4*	5.1 ± 0.6
Troponin I (ng/ml)	18.3 ± 30.1**	0.21 ± 0.61*	0	0
Coronary risk factors				
Hypertension (n, %)	73 (78)	63 (85)	56 (74)	0
Hypercholesterolemia (n, %)	43 (46)	36 (49)	37 (50)	38 (59)
Hypertriglyceridemia (n, %)	40 (43)	42 (57)	34 (46)	15 (23)
Diabetes mellitus (n, %)	28 (30)	28 (38)	43 (58)	0
Smoking (n, %)	47 (50)	29 (40)	45 (61)	20 (30)
Obesity (n, %)	25 (27)	6 (8)	16 (21)	11 (17)
Familial history (n, %)	44 (47)	30 (41)	34 (46)	43 (65)
Physical inactivity (n, %)	49 (53)	39 (43)	73 (98)	40 (61)
Statins (%)	48 (52)	41 (55)	58 (78)	23 (35)

Biochemical data were given as mean ± SD, * $p < 0.01$ versus control, ** $p < 0.001$ versus control

with amplification factor adjusted to 14. The baseline separation was achieved using a column Zorbax SB-C18 (150 × 4.6 mm, 3.5 μm) with a gradient between two mobile phase: A (sodium phosphate buffer, 40 mmol/L, pH 6.2) and B (deionised water:methanol; 1:9; v:v) within total run time of 10 min. The retention times for IS, ADMA and SDMA were 3.50, 3.85, and 4.10 min, respectively. Linearity, assessed by adding known amounts of dimethylarginines to the plasma sample in the final concentration range 0–1.25 μmol/L, yielded $r = 0.9905$ for ADMA and $r = 0.9935$ for SDMA. Analytical recovery resulted in 98 ± 4 and 94 ± 3.5 % (mean ± SD) for ADMA and SDMA, respectively. Precision was evaluated by five-time repeated analysis of the batched plasma sample and led <5 % (RSD) for each compound.

Serum glucose and troponin I concentrations were measured by standard laboratory methods on Olympus AU400 (Olympus, Tokio, Japan) and AxSYM (Abbott Ireland Diagnostics Division, Lisnamuck, Ireland), respectively.

Statistics

Most statistics were performed using the SPSS (the Statistical Package for Social Sciences) computer program. Mean, standard deviation, and median (minimum–maximum) were determined. Since the results of plasma ADMA and SDMA concentrations are not normally distributed, they are presented as Median (Min–Max). Mean and standard deviations of these markers are used for graphic presentation for practical reasons. The comparison of the studied groups was performed using ANOVA followed by Student's unpaired and paired t test. The clinical accuracy of the examined parameters was assessed using ROC curve analysis. ROC plots were constructed, and the areas under the curves (AUC), standard errors, 95 % confidence interval, sensitivity, specificity, optimal cutoff as well as positive and negative likelihood ratios (LR+, LR−) were calculated using the MedCalc computer program. The comparisons of the areas under different ROC plots were made using univariate z scores. A correlation analysis was performed using Pearson's correlation.

Results

Demographic and biochemical characteristics and risk factors in studied patients and controls are presented in Table 1. Coronary risk factors included: hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, smoking, obesity, familial history and physical inactivity. Risk factors were present in 8–98 % of the patients. Most often we noted hypertension, but others, except for obesity, were also observed very frequently in 30–98 % of the patients.

Plasma ADMA concentrations were 0.31 (0.17–0.87) μmol/L in control subjects, 0.76 (0.31–2.73) μmol/L in SAP patients, 0.94 (0.34–3.13) μmol/L in USAP patients, and 0.98 (0.48–2.01) μmol/L in AMI patients. All three patient groups showed significantly higher ADMA plasma levels than controls ($p < 0.0001$). No difference was found between patient groups (Fig. 1).

Plasma SDMA concentrations also were significantly higher in patient groups in comparison with healthy controls ($p < 0.0001$). In the control group, plasma SDMA levels were 0.29 (0.20–0.83) μmol/L, in the SAP group

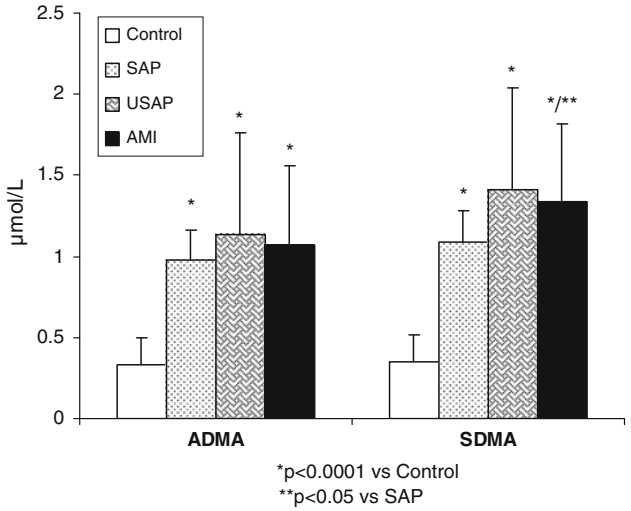

Fig. 1 Plasma ADMA and SDMA concentrations in patients with ischemic heart disease Graphically the results are presented as mean ± SD

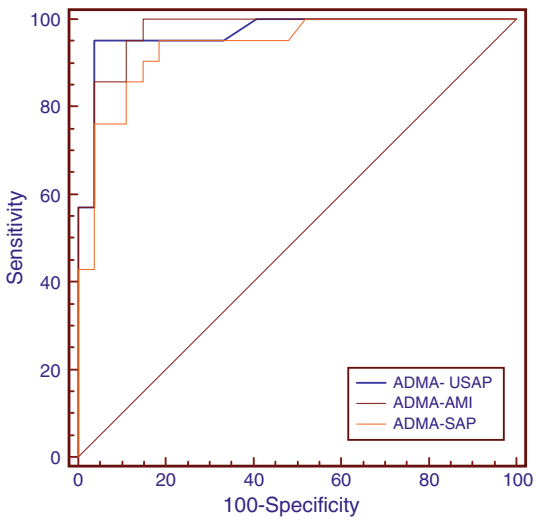

Fig. 2 ROC curve of ADMA in patients with ischemic heart disease

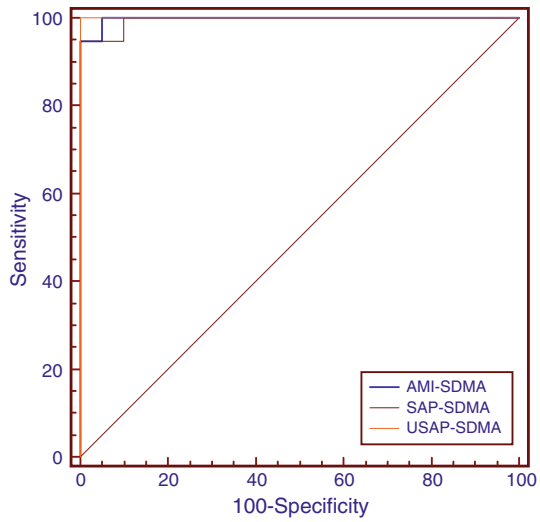

Fig. 3 ROC curve of SDMA in patients with ischemic heart disease

they were 1.11 (0.69–1.42) µmol/L, in the USAP group 1.23 (0.88–4.72) µmol/L, and in the AMI group 1.26 (0.75–2.93) µmol/L. Contrary to the ADMA levels, SDMA levels were significantly different between the SAP group and the AMI group ($p < 0.05$) (Fig. 1). Also, SDMA levels significantly negatively correlated with the serum creatinine concentration in healthy subjects ($r = -0.4$, $p < 0.01$), while in the NSAP patients this correlation was positive ($r = 1.0$, $p < 0.001$).

The ROC curves for ADMA and SDMA were presented in Figs. 2 and 3. The results of the ADMA ROC curve analysis (Table 2) showed that all three patient groups had AUCs above 0.9. The highest AUC obtained in AMI patients was 0.972, the similar AUC (0.971) was obtained in USAP patients, and to some degree lower in SAP patients (0.937). There was no significant difference between these three AUCs. The confidence interval was between 0.828 (in the SAP group) and 0.998 (in the USAP and AMI groups). We also observed very high ADMA sensitivity and specificity for determined cutoff values. The greatest sensitivity (95.7 %) and specificity (96.3 %) were found in the USAP group, in the AMI group, these characteristics were 100 % and 85.2 %, while in the SAP group 95.2 and 81.5 %, respectively. High positive likelihood ratios (LR+) and very low negative likelihood ratios (LR–) were noted for ADMA, especially in the USAP group (25.83). Much higher LR+ and LR− were obtained for SDMA patient values.

The analysis of the ROC curve for SDMA (Table 2) showed slightly better characteristics. The AUCs were between 0.995 (in SAP patients) to 1.000 (in the USAP group). Similar sensitivity (94.7 %) for ADMA and higher specificity (100 %) were obtained for SDMA in the SAP patients. In the USAP group, both these characteristics were 100 %, while in the AMI group they were 100 and 95 %, respectively.

A significant positive correlation between ADMA and SDMA was found in healthy individuals ($r = 0.259$, $p < 0.01$). In patients, a significant correlation between studied markers was observed only in the SAP group ($r = 0.196$, $p < 0.05$). Smoking was found to be the only risk factor showing a significant negative correlation with both markers in all patient groups. In the SAP patients, ADMA also significantly correlated with hypertension, cholesterol, triglycerides, positive familial history, physical inactivity and statin treatment. In the USAP patients, there was a significant correlation between ADMA and diabetes mellitus, and in the AMI patients with ADMA and CRP,

Table 2 The results of ROC curve analysis of ADMA and SDMA in patients with ischemic heart disease

	AUC	SE	95 % CI	Sensitivity	Specificity	Cutoff	LR+	LR−
SAP								
ADMA	0.937	0.006	0.828–0.987	95.2	81.5	>0.39	5.14	0.058
SDMA	0.995	0.006	0.900–1.000	94.74	100	>0.83	94.74	0.053
USAP								
ADMA	0.971	0.000	0.872–0.998	95.7	96.3	>0.61	25.83	0.045
SDMA	1.000	0.000	0.910–1.000	100	100	>0.83		
AMI								
ADMA	0.972	0.003	0.878–0.998	100	85.2	>0.41	6.75	0.000
SDMA	0.997	0.003	0.905–1.000	100	95	>0.69	20	0.0

Table 3 Correlation between ADMA/SDMA and patient risk factors

	AMI	USAP	SAP
CRP			
ADMA	$r = 0.294$, $p < 0.01$	NS	NS
SDMA	NS	$r = 0.693$, $p < 0.01$	NS
Hypertension			
ADMA	NS	NS	$r = 0.331$, $p < 0.01$
SDMA	NS	$r = -0.352$, $p < 0.01$	$r = 0.410$, $p < 0.01$
Cholesterol			
ADMA	$r = 0.183$, $p < 0.05$	NS	$r = 0.236$, $p < 0.01$
SDMA	NS	NS	$r = -0.293$, $p < 0.01$
Triglycerides			
ADMA	NS	NS	$r = 0.329$, $p < 0.01$
SDMA	NS	NS	NS
Diabetes mellitus			
ADMA	NS	$r = 0.476$, $p < 0.01$	NS
SDMA	$r = -0.195$, $p < 0.05$	NS	NS
Smoking			
ADMA	$r = -0.171$, $p < 0.05$	$r = -0.295$, $p < 0.01$	$r = -0.344$, $p < 0.01$
SDMA	$r = -0.354$, $p < 0.01$	$r = -0.267$, $p < 0.01$	$r = -0.212$, $p < 0.05$
Heredity (+)			
ADMA	NS	NS	$r = -0.243$, $p < 0.01$
SDMA	NS	NS	NS
Physical activity			
ADMA	NS	NS	$r = -0.695$, $p < 0.001$
SDMA	NS	NS	NS
Statins			
ADMA	NS	NS	$r = -0.281$, $p < 0.01$
SDMA	$r = -0.215$, $p < 0.05$	NS	$r = -0.612$, $p < 0.01$

cholesterol and triglycerides. Plasma SDMA levels showed a significant positive correlation with hypertension and negative with cholesterol and statins in the SAP group. In the USAP group, a positive correlation was found with CRP, but negative with hypertension, while in the AMI patients SDMA negatively correlated with diabetes and statins (Table 3).

Discussion

The main finding of the present study was that both plasma ADMA and SDMA concentrations had high clinical accuracy assessed by ROC curve analysis in patients with different stages of ischemic heart disease. Both markers showed up to four times higher levels in patients than in

healthy individuals. The control values were similar to those recently published (Hov et al. 2007; Meinitzer et al. 2007; Schwedhelm et al. 2009) in various European populations determined by the same method. Schwedhelm et al. (2009) defined the 95 % ADMA reference interval from 0.311 to 0.732 μmol/L in a reference sample without cardiovascular disease, diabetes, obesity, smoking or hypertension. They also showed that ADMA plasma concentrations increased by 0.0017 μmol/L for each year increase in age after adjusting for other variables in the model, while Hov et al. (2007) observed that age affected ADMA in healthy women, but not in men.

It is known that plasma ADMA levels are increased in people with hypercholesterolemia, atherosclerosis, hypertension (Surdacki et al. 1999), chronic heart failure (Sibal et al. 2010), diabetes mellitus associated with early diabetic nephropathy in Type 1 diabetes (Tarnow et al. 2004), but ADMA levels are not associated with endothelial dysfunction in young adults with Type 1 diabetes without microalbuminuria or known macrovascular disease (Sibal et al. 2009). Both ADMA and SDMA are significantly elevated in patients with chronic renal failure, where the increase was more pronounced for SDMA (Fleck et al. 2001, 2003). We also found a highly significant positive correlation between serum creatinine concentration and SDMA levels in patients with USAP. The increasing serum SDMA concentration seems to play an additional role in the renal outcome besides serum creatinine and hemoglobin levels (Busch et al. 2006).

Several studies have shown that ADMA was a novel and an independent cardiovascular risk factor (Schulze et al. 2006) as well as an independent predictor of fatal and nonfatal myocardial infarction and stroke in women (Leong et al. 2008). ADMA is a strong predictor of cardiovascular events and death in selected patient populations (Fliser et al. 2005) and a marker of the progression of various chronic renal diseases (Kielstein and Zoccali 2005; Ravani et al. 2005), while serum SDMA concentrations are independently associated with increased cardiovascular and total mortality in patients undergoing coronary angiography (Meinitzer et al. 2011; Schulze et al. 2010). The ADMA level of 0.71 μmol/L or higher gives a relative risk of future myocardial infarction and stroke events of 1.75 and a population attributable risk of 12.7 % (Leong et al. 2008). These authors suggested that the concentration of 0.71 μmol/L might be a clinically useful cut point. The results of ROC curve analysis for ADMA in our patients showed that the optimal cutoff value in the SAP patients was >0.39 μmol/L, in the USAP patients it was >0.61 μmol/L, and in the AMI group >0.41 μmol/L, with the diagnostic values of 0.937, 0.971, and 0.972 in SAP, USAP and AMI patients, respectively. However, we also showed that SDMA, mainly observed as a kidney marker, had a higher optimal cutoff value >0.83 μmol/L in both SAP and USAP patients, and >0.69 μmol/L in the AMI patients, and higher diagnostic values of 0.995, 1.000, and 0.997 in the relative groups of patients. The highest sensitivity (100 %) and specificity (100 %) obtained in the USAP group showed that for the cutoff value of >0.83 μmol/L SDMA might be a useful marker in assessing disease activity in 100 % patients. These findings showed that SDMA would be a marker with better clinical accuracy in all stages of ischemic heart disease, particularly in patients with USAP, despite the fact that neither ADMA nor SDMA AUCs showed significant differences between the patient groups. High positive likelihood ratios and very low negative ones for both markers suggested that ADMA and SDMA might be clinically relevant in assessing ischemic disease.

By testing an interdependence in the studied markers and traditional risk factors, we found different correlation patterns for ADMA and SDMA in various stages of ischemic heart disease. Both markers had a significant negative correlation with smoking in all patient groups. Contrary to the results of Päivä et al. (2003), we obtained a significant negative correlation between ADMA and statins in the SAP group and between SDMA and statins in the SAP and the AMI groups. Plasma ADMA concentrations correlated with CRP in patients with peripheral artery disease, while homocysteine-lowering therapy does not affect plasma ADMA levels (Ziegler et al. 2005). These results show that the disturbance in the metabolism of dimethylarginines is associated with almost all classical risk factors as well as therapeutic drugs.

A mechanism leading to the increase in ADMA concentration in ischemia–reperfusion injury was studied in animal models. In a genetic mouse model, Stuhlinger et al. (2007) observed that after ischemia followed by reperfusion exactly 30 min later the maximum increase in tissue ADMA reached at 4 h of reperfusion coincided with the reductions of nitric oxide concentration and DDAH activity. Furthermore, they found that DDAH over expression and the treatment with L-arginine markedly reduced reperfusion injury by 40–50 % at 4 h of reperfusion. Similar results were published recently (Blokhin et al. 2009) showing the elevation of ADMA by 30 % in plasma and 50 % in the heart of DDAH deficient mice after ischemia–reperfusion injury. These results show that an impairment of DDAH is a significant determinant of reperfusion injury, a mechanism that is independent of reperfusion-induced oxidant stress. The absence of correlation between ADMA and SDMA in the USAP and AMI patients observed in this study is in agreement with cited results. Our results related to plasma ADMA and SDMA concentrations showing a significant increase in these markers independently of the stage of ischemic heart

disease confirm that an increase in dimethylarginines may be considered as a marker of ischemia.

Conclusion

To summarize, our data suggest that ischemic heart disease is associated with a significant increase in plasma ADMA and SDMA concentrations independently of the disease activity. Although both markers show high clinical accuracy, we observed better characteristics for SDMA. These results prove SDMA as a valid marker and a therapeutic target in ischemic heart disease.

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